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Structuring Amazonian bat assemblages: importance of horizontal and vertical dimensions of habitat

Doutoramento em Biologia

Especialidade de Ecologia

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Tese orientada por:

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Documento especialmente elaborado para a obtenção do grau de doutor

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Lisboa, março de 2016

João Tiago Sabino Lino Marques

Para Neia, Carolina, Pedro e Joana

Aos meus pais, Amélia e João, e irmã, Helena

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"Há outro rio chamado Maranhão, que desagua no Rio Tocatins, como diremos em seu lugar; mas ainda que é grande e caudaloso, é regato a respeito deste Maranhão; Orelhana ou Amazonas, que pela sua grandeza, longitude, e muitas águas, se faz digno de ser chamado um mar natante, o máximo e monarca dos rios, e merecedor de muitos nomes e muitos títulos."

Padre Jesuíta João Daniel

O Tesouro Descoberto no Rio Amazonas sem data

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Abstract

Environmental heterogeneity is one of the factors that underpins the species richness of an area. However, this important relationship has only been poorly documented in tropical regions or in low human impact areas due to the scant data. The overall objective of this thesis is to contribute to the understanding of how the spatial components of environmental heterogeneity, both horizontal and vertical, contribute to the high number of ecological niches that explain the high biodiversity of Amazonian rainforests. More specifically, this thesis attempts to unveil the relevance of different types of forests, river bank vegetation and forest vertical component to the ecological structure of the megadiverse bat fauna of Amazonia.

First, in Chapter 2 we focused on the horizontal component of environmental heterogeneity. In this paper we studied forest inundation and river banks as determinants of the spatial variation in the availability of fruits for bats in lowland Amazonia. We sampled the bat assemblage composition, fruit availability, and bat diet in terra firme upland forest and in two types of flooded forest - várzea and igapó. We grouped the bat species in two functional groups, the understorey and the canopy feeding guilds. We found that the várzea forests had abundant fruit resources for the canopy bats, while terra firme provided more fruits for the understorey bats. Additionally, river bank vegetation provided more fruit availability for bats, particularly for the canopy feeding guild on the edges of the várzea forests and for the understory feeding guild on the edges of the terra firme forests.

Moreover, bats showed high fidelity to their feeding guild; they consumed a high proportion of their preferred fruits - canopy or understory - even in forest types or areas where these fruits were scarce.

In conclusion, both the forest type and the river bank vegetation influence the availability of fruits, although differently for the canopy and understory guilds. The resulting differences in fruit abundance may explain variations in bat assemblages of the different forest types. River banks play a particularly important role in providing food for bats of both guilds. By increasing the niche space, the mosaic of forests may play an important role in supporting such a diverse bat assemblage.

The assessment of the contribution of the vertical component of environmental heterogeneity to the structuring of the Amazonian bat assemblage is presented in Chapter 3. Here, we studied the vertical space use of aerial insectivorous bats. Using automatic ultrasound recording stations placed in the canopy, subcanopy and understorey we tested if bat activity and species diversity are vertically stratified, both in the forest interior and near the edges of water bodies. These patterns were tested separately for 21 individual species, and for two functional groups - open space and edge space bats. Our results show that insectivorous bat activity increased by roughly seven fold, and species diversity doubled, from the understorey to the canopy. Both edge space and open space bats were more active in the upper strata, but this tendency was much more accentuated in the latter. *Myotis riparius* was the only species with greater activity near the understorey. These broad patterns were altered at the edges of water bodies, where vertical stratification was much less marked.

The observed patterns are parsimoniously explained by constraints imposed by vegetation clutter that change across strata, which affect bat species differently. Only bats better adapted to closed spaces are usually capable of foraging within the understorey, whereas the majority of species can exploit the free spaces immediately below the canopy; open space bats seem to concentrate their activity above the canopy. Our results underline the need to preserve pristine stratified rainforests. Moreover, the concentration of insectivorous bats at the upper strata of rainforests underlines the need to include canopy level sampling in ecological studies.

The study of the pitfalls and optimization of survey methods is one of the important tasks of scientists. In Chapter 4 we addressed this concern and investigated the impact of mist net avoidance in bats and birds. We quantified the day-to-day decline in captures of Amazonian birds and bats with mist nets set at the same location for four consecutive days. We also evaluated how net avoidance influences the efficiency of surveys under different logistic scenarios by subsetting data. Our results demonstrated that net avoidance caused substantial declines in bird and bat captures, although more accentuated in the latter. Most of the decline occurred between the first and second days of netting: 28 % in birds and 47% in bats. Captures of commoner species were more affected and the numbers of species detected also declined. Moving nets daily to minimize the avoidance effect increased captures by 30% in birds and 70% in bats. However, moving the location of nets may cause a reduction in netting time and captures, and we further investigated the implications of that logistic constraint. When moving the nets caused the loss of one netting day it was no longer advantageous to move the nets frequently. In bird surveys that could even decrease the number of individuals captured and species detected.

Net avoidance can greatly affect sampling efficiency but adjustments in survey design can minimize this. Whenever nets can be moved without losing netting time and the objective is to capture many individuals, they should be moved daily. If the main objective is to survey species present then nets should still be moved for bats, but not for birds. However, if relocating nets causes a significant loss of netting time, moving them to reduce effects of shyness will not improve sampling efficiency in either group. Overall, our findings can improve the design of mist netting sampling strategies and can be applied in other tropical areas.

The novel information presented in this thesis and the conclusions indicate that both the horizontal and vertical components of habitat heterogeneity are very important to increase niche space. However, I propose to add a new spatial component, the habitat

disruptions or discontinuities, to the horizontal and vertical components of environmental heterogeneity. This component, which integrates both the river bank vegetation and the free spaces between the vertical forest layers, has a similar relevance than the two components; it plays a significant role in the link between environmental heterogeneity and species richness.

Key-words: environmental heterogeneity, flooded and unflooded forests, river bank vegetation, vertical stratification, Amazonian bats

Resumo

A diversidade de espécies animais está directamente relacionada com as características ambientais. Vários trabalhos de investigação têm focado este tema de investigação, mas existem poucos estudos em áreas tropicais e em regiões com muito baixo impacto humano. O objetivo geral desta tese é contribuir para o conhecimento de como os componentes espaciais da heterogeneidade ambiental, o componente horizontal e o vertical, contribuem para o elevado número de nichos ecológicos que explicam a elevada biodiversidade das florestas Amazónicas. Especificamente, será avaliada a relevância dos diferentes tipos de floresta, da vegetação ripícola e da estratificação vertical para a estruturação da fauna hiperdiversa de quirópteros da Amazónia.

No capítulo 2 são apresentados os resultados da investigação sobre a componente horizontal da heterogeneidade ambiental. Neste artigo foi estudado o efeito da inundação das florestas e da vegetação das margens dos cursos de água na variação espacial da disponibilidade de frutos para os morcegos nas florestas da bacia do Amazonas. Amostrámos a composição das espécies de morcegos, a disponibilidade de frutos e a dieta dos morcegos na floresta não inundável, terra firme, e em dois tipos de florestas inundáveis, a várzea e o igapó. Também foram amostrados locais de vegetação da margem de cursos de água na orla dos três tipos de florestas. As espécies de morcegos foram classificadas em dois grupos funcionais, os morcegos que se alimentam de frutos da copa e os que se alimentam de frutos presentes em arbustos e pequenas árvores no subcoberto. Os resultados indicam que as florestas de várzea têm maior abundância de recursos para os morcegos de copa, enquanto a terra firme tem maior disponibilidade de frutos para os morcegos que se alimentam no subcoberto. Ademais, os morcegos demonstraram uma elevada fidelidade ao seu grupo funcional; eles consumiram uma elevada proporção dos frutos

preferidos – de copa ou de subcoberto - mesmo nas áreas ou tipos de floresta em que estes são escassos. A vegetação de orla dos cursos de água apresentou maior disponibilidade de frutos de copa nas florestas de várzea e de frutos de subcoberto, nas florestas de terra firme.

Concluímos que ambos os fatores, o tipo de floresta e a vegetação ripícola influenciam a disponibilidade de frutos, embora de modo diferente para os grupos de morcegos que se alimentam de frutos de copa ou que se alimentam de frutos de subcoberto. Estas diferenças podem explicar as variações da ocorrência e abundância dos morcegos frugívoros nos diferentes tipos de floresta. A vegetação ripícola desempenha um papel muito importante na disponibilidade de alimento para os morcegos de copa nas áreas ribeirinhas de florestas de várzea e para os morcegos que se alimentam no sub-coberto nas áreas ribeirinhas de florestas de terra firme. O mosaico com florestas de terra firme, igapó e várzea aumenta o número de nichos ecológicos e pode desempenhar um papel importante no suporte a uma comunidade de morcegos com elevada diversidade.

A influência da componente vertical da heterogeneidade ambiental para a estruturação da comunidade de quirópteros Amazónica foi avaliada estudando o uso do espaço vertical por morcegos insectívoros que capturam os insetos em vôo (Capítulo 3). Utilizando estações de gravação automática de ultra-sons colocadas a diferentes alturas na floresta, na copa, na subcopa e no subcoberto, nós testámos se a atividade dos morcegos e a diversidade de espécies têm uma estratificação vertical, tanto em áreas de floresta como na orla de cursos de água e lagos. A existência destes padrões foi testada separadamente para as 21 espécies e para dois grupos funcionais – morcegos que caçam em espaços abertos e morcegos que caçam em orlas.

A atividade dos morcegos insetívoros aumentou aproximadamente sete vezes e a diversidade de espécies duplicou comparando os resultados de copa com os recolhidos no sub-coberto. Tanto os morcegos que caçam em áreas abertas como os que caçam nas orlas estiveram mais activos nos estratos superiores, mas esta tendência foi muito mais acentuada nos últimos. A espécie *Myotis riparius* foi a única com maior atividade no estrato inferior. Estes padrões não foram observados nas margens dos corpos de água, onde a estratificação vertical é menos marcada.

Os padrões observados são explicados parcimoniosamente pelos constrangimentos impostos pela densidade de obstáculos da vegetação, que muda consoante os estratos, e que afecta as espécies de morcegos de modo diferente. Apenas os morcegos que estão melhor adaptados a voar em espaços fechados são capazes de procurar alimento no sub-coberto, enquanto a maioria das espécies se alimenta nos espaços livres debaixo da copa; os morcegos que voam nos espaços abertos concentram a sua atividade acima da copa da floresta. Os resultados sublinham a importância da preservação de florestas pristinas e com vegetação estratificada. Ademais, a concentração de morcegos insetívoros nos estratos superiores das florestas indica que devemos incluir a amostragem de copa nos estudos ecológicos.

O estudo das desvantagens e a otimização dos métodos de amostragem são tarefas importantes para os cientistas que as usam. No capítulo 4 são apresentados os resultados do impacto do comportamento de evitar as redes nos morcegos e nas aves. Este comportamento verifica-se quando os animais aprendem onde estão localizadas as redes de captura e começam a evitar-las. Neste artigo nós quantificámos a diminuição das capturas de morcegos e aves da Amazónia quando as redes japonesas ficam colocadas no mesmo local por quatro dias consecutivos. Também foi avaliado como o comporetamento de evitar as redes pelos animais influencia a eficiência das inventariações biológicas em diferentes cenários logísticos.

O comportamento de evitar as redes causou diminuições substanciais nas capturas de aves e de morcegos, embora seja mais acentudada nos últimos. A maior parte do declínio ococrreu entre o primeiro e o segundo dia de amostragem: 28% nas aves e 47% nos morcegos. As capturas das espécies mais comuns foram que mais decairam e o número de espécies também diminuiu.

Mover as redes a cada dia para minimizar o comportamento de evitar as redes aumenta as capturas em 30% para as aves e em 70% para os morcegos. Contudo, ao mover as redes de local podemos gastar tempo de amostragem. Se quando movemos as redes perdemos um dia de amostragem pode não ser vantajoso mover as redes frequentemente. Nos trabalhos de inventariação de aves isso até pode reduzir o número de indivíduos capturados e de espécies detectadas. Nós testámos as implicações destes efeitos contrários considerando diferentes cenários logísticos para aconselhar que medidas tomar para diminuir o impacto deste comportamento nos inventários biológicos.

O comportamento de evitar as redes pode ter um grande impacto na eficiência de amostragem mas ajustes no desenho de amostragem podem minimizá-lo. Quando as redes podem ser movidas sem perda de tempo de amostragem e o objectivo é a captura do maior número de indivíduos, devemos muda-las diariamente. Se o objectivo é a detecção do maior número de espécies numa área então as redes devem ser deslocadas diariamente para os morcegos, mas não para as aves. Contudo, se a mudança das redes causa uma perda de tempo de captura, então a mudança de local das redes não melhora a eficiência de amostragem nos dois grupos. As nossas conclusões podem ajudar o planeamento de estratégias de amostragem com redes noutras florestas tropicais.

As informações recolhidas e conclusões que integram esta tese indicam a elevada importância da componente horizontal e vertical para a relação entre a

heterogeneidade ambiental e a riqueza de espécies de morcegos. Ademais, devido ao papel importante da vegetação da orla dos cursos e corpos de água e dos espaços entre os estratos de vegetação vertical, é proposta a adição de um novo componente espacial, as descontinuidades do habitat. Este componente espacial tem uma importância semelhante ao componente horizontal e vertical na ligação entre a heterogeneidade ambiental e a riqueza de espécies.

Os resultados desta tese também apontam para a importância da conservação de florestas tropicais húmidas para a manutenção da elevada riqueza de espécies de morcegos. Estas florestas devem ser conservadas com a manutenção de mosaicos de diferentes tipos de floresta (terra firme, várzea e igapó), e com uma copa alta e bem preservada, mantendo os espaços livres por baixo da copa. Também é importante a preservação da vegetação de orla de cursos de água que interrompem as florestas Amazónicas, porque proporcionam recursos alimentares para morcegos frugívoros e áreas de alimentação com menor densidade de obstáculos para os morcegos insetívoros.

Palavras-chave: heterogeneidade ambiental, florestas inundáveis e terra firme, vegetação ripícola, estratificação vertical, morcegos da Amazónia

Chapter 1

General Background

1 General Background

Why there is this number of species in a habitat? What features make a region or a habitat able to host such a large or low number of species? These simple questions related to the conditions and drivers of the coexistence of species have been central issues in ecology (*e. g.* Hortal *et al.* 2009).

Environmental diversity, at multiple scales, is widely recognized as an important determinant of the number of species in ecosystems. Early work by Koopman (1958) stated the importance of "the element of ecological difference" influencing the total number of species inhabiting islands off the coast of South America. Later, Lack (1969) acknowledged that ecological constraints explain the small numbers of species on islands: islands with fewer habitats compared to nearest mainland area, *i. e.* "degree of ecological impoverishment", hosted less species. And, in their influential work, MacArthur and Wilson (1967) concluded that environmental diversity exerts a more direct influence than area on the species numbers present on islands.

Numerous empirical and theoretical studies described and tested the relationship between environmental diversity and species numbers (reviewed in Tews *et al.* 2004, Hortal *et al.* 2009). Recently, a robust meta-analysis, integrating data from a very large number of published empirical studies (n=1148 data points), found substantial quantitative support for a strong positive relationship between environmental diversity and species numbers (Stein *et al.* 2014). The authors concluded that this relationship is widespread across the world, is present in many different taxa, and is evident across multiple spatial scales, from small patches within habitats ($1m^2$ plots) to entire regions.

The mechanisms underlying the environmental heterogeneity-species diversity relationship can be divided into ecological and evolutionary categories (Stein and Kreft

2014), which can be further divided into three major groups acting at different time scales: ecological factors, species persistence promotion and speciation events. Ecological mechanisms have been recognized for long as a major underlying factor supporting the relationship between environmental heterogeneity and species richness diversity (Tews *et al.* 2004, Hortal *et al.* 2009, Brown 2014, Stein *et al.* 2014). The most common explanation is that environmental heterogeneity increases the number of habitats, the number and extent of environmental gradients, the availability of resources and the structural complexity, thus supporting an increase of niche space that enables more species to coexist (*e. g.* Tews *et al.* 2004). Therefore, heterogeneous habitats, landscapes or biomes provide more opportunities for species to coexist through more potential niches and diverse resources (Stein and Kreft 2014). Evolutionary mechanisms also play a role in the environmental heterogeneity-species diversity relationship, but on longer time scales; species experiencing adverse environmental conditions are more likely to persist in environmentally heterogeneous landscapes. Speciation events that result from the adaptation to different environmental conditions are also more likely to occur in areas with higher heterogeneity (Stein *et al.* 2014).

Environmental heterogeneity (EH) can be gauged using biotic and non-biotic measures with different degrees of success. In their comprehensive review, Stein *et al.* (2014) show that biotic measures of EH correlate with species richness better than non-biotic measures of EH such as climatic or soil variables. Moreover, among the most used biotic EH measures, plant diversity and vegetation complexity have the strongest correlation with animal species richness. They conclude that vegetation heterogeneity is a more direct driver of species richness, particularly for herbivores, because it provides a variety of food resources, shelter and roosting opportunities. Vegetation heterogeneity measures are also related to species richness of non-

herbivores because of their reliance on vegetation features that add structure to the habitats (Stein *et al.* 2014).

The overall positive EH-species diversity association (92.7% of statistically significant relationships) was present at several spatial scales, although the effect was stronger at smaller than at larger spatial scales (Stein *et al.* 2014). Surprisingly, the few negative EH and species diversity relationships also occurred mostly at small spatial scales. Confounding factors, such as fragmentation, may explain these outliers because they can be harder to account for at small spatial scales (Tews *et al.* 2004).

Despite of the remarkable number of empirical studies and meta-analyses on the relationship between species richness and EH, there are biases on the information collected. Most studies focus on vertebrate responses and, more importantly, take place on landscapes influenced by anthropogenic activity (Tews *et al.* 2004). As referred before, in such landscapes, environmental heterogeneity can even be confused with factors like fragmentation and habitat degradation (Tews *et al.* 2004). Therefore, there is a need for further empirical research on how EH relates to species richness in well-preserved landscapes, especially at small spatial scales.

At smaller spatial scales, such as habitats or within habitats, EH has two distinct dimensions: the horizontal component of variation (habitat heterogeneity) and the vertical component of variation (habitat complexity or vertical stratification) (sensu August 1983). Thus, throughout the text I employ the terms (Stein *et al.* 2014): (i) habitat diversity referring to the number of habitat types in a region, (ii) habitat heterogeneity for the horizontal variation within-habitat and (iii) habitat complexity to address within-habitat vertical variability, particularly its vertical stratification (August 1983, Hortal *et al.* 2009).

The contribution of the horizontal and vertical components of EH to overall species richness diversity, probably varies among biomes. Fahr and Kalko (2011)

recognized this and conceptualized a theoretical model of the influence of both components for the species diversity across a biome transect. According to their model, in rainforests habitat complexity (vertical component) has a higher contribution to species diversity than habitat heterogeneity (horizontal component) (Fig. 1). The authors base this model on the empirical observations that rainforest canopies consistently form a homogeneous habitat; across extensive rainforest regions, the dense canopy of rainforests is only interrupted by tree gaps and water courses. Although the model was put forward for West African forests, it seems to be adequate to the Amazonian rainforests. In fact, in the Amazon basin the numerous watercourses are the most prevalent interruption in the forest canopy. The riverbank vegetation present along many of these watercourses may thus represent a most important component of the horizontal heterogeneities in Amazonian rainforest (Junk and Piedade 2010). Moreover, within the large rainforest matrix, habitat complexity - the vertical structure of the vegetation - should play the most important role in increasing heterogeneity and the availability of ecological niches for species.

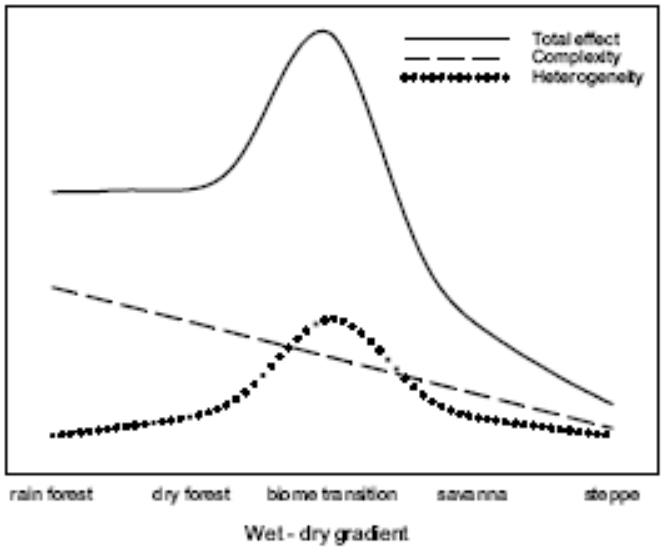

Figure 1.1 Hypothetical model of the contribution of habitat structure on species richness along a biome gradient from rainforest to steppe. The contribution of habitat complexity, *i. e.* vertical component, on species richness is highest in rainforest (adapted from Fahr and Kalko 2011).

Tropical rainforests are the terrestrial biome that holds the highest species richness. It has been suggested that this incredible biodiversity is possible because the warm moist environment offers relatively benign abiotic conditions and abundant resources (Brown 2014). Almost half of the tropical rainforests are in the Neotropics, which also hold the world's largest single block of rainforests: the Amazonian rainforest, covering basins of the Orinoco and the Amazon rivers and extending to the Guyanan shield (Corlett and Primack 2011). This extensive region holds an incredible biodiversity, and for several plant and animal groups, such as butterflies, birds and bats, this is the place on Earth where we can find the highest number of co-existing species (Corlett and Primack 2011).

In the Amazonian forests, bats make up a very high proportion of the vertebrate biomass; in some regions they constitute up to 60% of the mammalian fauna (Eisenberg 1990, Simmons and Voss 1998). Bat species richness in Amazonian sites often exceeds 70 species (Simmons and Voss 1998, Lim and Engstrom 2001, Bernard

and Fenton 2002, Sampaio *et al.* 2003) and it is estimated that the Amazon basin hosts 117 bat species (Marinho-Filho and Sazima 1998).

Bats' functional diversity also peaks in the Amazonian forests. They occupy as many as 10 feeding guilds (Kalko 1998), including some particularly well-known from this region, such as sanguivores and obligate piscivores. Both their impressive species richness and functional diversity contribute to the ecological importance of bats in the Amazonian forests. Throughout the Neotropical region they play very important ecological roles as plant-pollinators (Bawa 1990), seed dispersers over large distances and across open habitats (Muscarella and Fleming 2007), and controllers of insect herbivory (Kalka *et al.* 2008, Williams-Guillén *et al.* 2008).

Bats' flight capacity enables them to explore rainforest niches that other vertebrates cannot reach, potentially taking full advantage of the niche spaces created by both habitat heterogeneity and complexity (sensu August 1983). They can easily travel to and forage in habitats and patches located several kilometers across the landscape (Morrison 1978, Ramos Pereira *et al.* 2009, Bobrowiec *et al.* 2014). Bats can also exploit several forest height strata, from ground level to over the forest canopy (Bernard 2001, Kalko and Handley 2001, Ramos Pereira *et al.* 2010).

Amazonian bats and birds share characteristics that are important determinants of their use of ecological space. Both groups are very speciose and have the ability to fly, which allows them to exploit extensive areas of habitat. Because of these similarities various studies compared the organization of both assemblages, their ecological needs and foraging ecologies (*e. g.* Palmeirim *et al.* 1989, Kalko 1998). These studies often use similar techniques to study bats and birds in rainforests, particularly when data collection requires capturing individuals. Mist-netting is one of the essential techniques to study the diverse assemblages of both Neotropical bats and birds. It is one of the most widely used techniques to capture and collect data for

biodiversity and auto-ecological studies for these two groups of flying vertebrates (Beja *et al.* 2010, Blake and Loiselle 2009, Ramos Pereira *et al.* 2009, Meyer *et al.* 2011, Bobrowiec *et al.* 2014). However, there is an increasing demand for empirical studies and fieldwork to be cost-effective and for that reason we need to assess the efficiency of the research tools and techniques that we use for collecting and analyzing data (Gardner *et al.* 2008, Meyer *et al.* 2015). Consequently, studies to optimize sampling methods in rainforests can make an important contribution to the advancement of knowledge of these ecosystems.

Despite the similarities in morphology of bats and birds there are also important ecological and functional differences in the Neotropics. Kalko (1998) reports 12 feeding guilds exclusive of birds and two feeding guilds exclusive of bats, although one third of the total guilds analysed were common to the two taxa. Bats and birds also differ in their response to land use change such as habitat fragmentation or degradation (Barlow *et al.* 2007). Neotropical bats respond to fragmentation in an idiosyncratic way, whereas birds behave as expected in a gradient of rainforest degradation. Bat species richness is higher in primary forests, but similar in secondary and plantation forests; bird species richness decreases monotonically from primary forests, to secondary forests and to plantations (Barlow *et al.* 2007).

Bats have very high energy demands because flying is costly (Kurta *et al.* 1989); birds have comparatively lower flight energy demands, because of their greater aerodynamic efficiency (Muijres *et al.* 2012). To meet these high energy needs bats need to find quickly and to forage in the most productive feeding areas, whether within habitats or across different habitats. Thus, the specific question “Where do bats forage?” is one of the most interesting and prevailing questions in the ecology of this group coupled with “What are the constraints or features of an area that is good bat foraging ground?”

1.1 Thesis objectives and organization

The overall objective of this thesis is to contribute to the understanding of how the vertical and horizontal components of habitat structure contribute to the high number of ecological niches that explain the tremendous biodiversity of Amazonian rainforests. More specifically, the thesis attempts to unveil the relevance of different types of forests, riverbank vegetation and forest vertical component to the ecology of the megadiverse bat fauna of Amazonia.

This thesis is structured in two main sections; the first analyses how both horizontal and vertical heterogeneity influence the structure of bat assembles in Amazonia, while the second contributes to the optimization of sampling of assemblages of flying vertebrates

The first section includes the results of two papers (Chapters 2 and 3). First, we investigated the contribution of horizontal environmental heterogeneity for species richness of fruit eating bats. To do that, I assessed the availability of fruits in seasonally flooded and unflooded forests – different habitats - and compared fruit availability between matrix forests with riverbank vegetation – habitat discontinuities.

Second, we investigated how bats fly and forage across the vertical strata of Amazonian forests. Specifically, we assessed how aerial insectivorous bats use the different height strata in the rainforests and how this use is altered by riverbank vegetation, which constitutes the most prevalent disruption of the dominant vertical stratification of these forests.

In the second section (Chapter 4) I present a third paper dealing with the limitations of mist-netting to collect data for auto-ecological and biodiversity field studies in Neotropical rainforest. Specifically, we assessed how net avoidance influences the number of captures of bats and birds and evaluate its impact on species richness estimates. The analyses for the two groups of flying vertebrates provide a comprehensive evaluation of the effect of this common response-behaviour to a widely used data collection technique.

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Chapter 2

Availability of food for frugivorous bats in lowland Amazonia: the influence of flooding and of river banks

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2 Availability of food for frugivorous bats in lowland Amazonia: the influence of flooding and of river banks

2.1 Abstract

In Neotropical forests fruits are key resources for a great diversity of vertebrates, including many frugivorous bats, but little is known about the factors that determine their availability. We studied forest inundation and river banks as determinants of the spatial variation in the availability of fruits for bats in lowland Amazonia. We sampled the bat assemblage composition, fruit availability, and bat diet in terra firme upland forest and in two types of flooded forest - várzea and igapó. Two distinct frugivore bat guilds were found in both terra firme and flooded forests: (1) canopy frugivores, feeding mainly on Ficus and Cecropia, and (2) understorey frugivores that feed mainly on Vismia and Piper. Fruits consumed by the canopy guild were more abundant in the flooded forests - particularly in the nutrient-rich várzea, but those dominating the understory guild diet were most abundant in terra firme. Availability of both fruit genera most consumed by the canopy guild was greater along river banks than in the forest matrix. For the understory guild, the greater abundance of Vismia along river banks was compensated for by a higher availability of Piper in the matrix. In conclusion, both factors influence the availability of fruits, although differently for the canopy and understory guilds. The resulting differences in fruit abundance may explain variations in bat assemblages of the different forest types. River banks play a particularly important role in providing food for bats of both guilds, but are under particularly heavy human pressure.

2.2 Introduction

Most trees and shrubs of tropical rainforests produce fleshy fruits (Jordano, 2000), which are important and abundant food resources consumed by a diverse array of vertebrates; in some Neotropical forests the bulk of the bird and mammal biomass is supported by fleshy fruits (Fleming *et al.*, 1987).

Bats constitute up to 60% of the Amazonian mammalian fauna, dominating the community in diversity and biomass (Simmons and Voss, 1998), and playing essential ecological functions. Frugivorous bats are particularly abundant in these Neotropical forests, where they are key seed dispersers of many plants that produce fleshy fruits (Giannini and Kalko, 2004; Lopez and Vaughan, 2004) and have an important role in forest regeneration and succession (Muscarella and Fleming, 2007; Willig *et al.*, 2007).

Bats have high energetic requirements because flight is very costly (Kurta *et al.*, 1989) and they have high metabolism, particularly in the case of frugivorous species (McNab, 1986). As the nutritional value of fruits tends to be low (Wendeln *et al.*, 2000), bats need to eat large amounts of food to satisfy these high requirements (Morrison, 1978). Flight facilitates the fulfilment of these requirements, as it allows the selection of high quality feeding areas (Law, 1995) and, in fact, foraging frugivorous bats tend to be most active in the most productive habitats and sites (Willig *et al.*, 2007; Ramos Pereira *et al.*, 2009; Vargas-Contreras *et al.*, 2009). Consequently, like for nectarivorous (Lemke, 1984) and insectivorous bats (Wang *et al.*, 2010), the quality of foraging patches may be one of the main factors driving foraging decisions of frugivorous bats.

In the lowlands of Central Amazon, frugivorous bats seem to make a distinct use of different existing forest types (Ramos Pereira *et al.*, 2009). Most of this vast

region is covered by terra firme forests that never flood, which are nutrient-poor because they have long been deprived of alluvial sediments (Irion *et al.*, 1997). However, approximately 10% of the region is dominated by two forest types that are flooded for much of the year: the nutrient-rich várzea, seasonally flooded by “white-waters” loaded with sediments from the ice-melt and steep slopes of the Andes, and the oligotrophic igapó, seasonally flooded by nutrient-poor “black-water”, stained by organic compounds and originating in the forest plains (Prance, 1979; Ayres, 1993).

Amazonian forests have a mostly continuous and dense canopy layer, but along water courses this layer is interrupted, allowing abundant light to reach the ground (Bongers *et al.*, 2001). This availability of light along the river banks and the proximity of water are likely to influence both the floristic composition of the forest and the fruiting phenology and productivity of its trees and shrubs (van Schaik *et al.*, 1993). Along river margins these plants face less competition for solar radiation than inside the forest (Bongers *et al.*, 2001), allowing them to allocate more energy to flower and fruit production (van Schaik *et al.*, 1993). Consequently, it is likely that plants in river banks provide resource-rich patches to frugivores, including bats. Moreover, the distinct floristic composition of river bank forest (*e. g.* Salo *et al.*, 1986) may diversify foraging opportunities for a variety of bat foraging guilds.

In lowland Amazonia, another factor potentially increasing fruit availability along river banks is their frequent natural disturbance due to lateral river erosion, and the subsequent formation of sediment beaches (Salo *et al.*, 1986; Peixoto *et al.*, 2009). These newly exposed soil and beaches are areas of primary succession (Parolin *et al.*, 2002; Myster, 2009), and many frugivorous bats may exploit the abundant fruit resources provided by some pioneer and early-successional plants that become established in them, such as Cecropia, Piper and Solanum (Fleming, 1988; Palmeirim *et al.*, 1989; Bernard, 2002; Aguiar and Marinho Filho, 2007).

This evidence suggests that in Neotropical rainforests vegetation along river banks may play a disproportionally important role in the provision of food resources for its rich fauna of frugivorous vertebrates, but to our knowledge this possibility has never been evaluated. There is also very little information about the relative availability of fruit in the different types of flooded and unflooded forests present in the central Amazonian floodplain, as only one paper presents data on this topic (Haugaasen and Peres, 2007).

In this study we assessed the potential value of river bank vegetation as foraging habitat for the diverse guild of Amazonian frugivorous bats, in lowland landscapes with flooded and unflooded forests. Our specific objectives were: (1) to study the feeding guild structure of an assemblage of frugivorous bats in a region dominated by a mosaic of terra firme, várzea, and igapó forests; (2) to identify differences in food availability in the three types of forest; and (3) to determine the potential importance of the vegetation along river banks to provide food for frugivorous bats.

2.3 Materials and Methods

Study Area

Field work took place in the Amanã Sustainable Development Reserve (ASDR, Amazonas, Brazil), a reserve covering 2 350 000 ha that contains nutrient-rich várzea forests and nutrient-poor igapó forests, within a matrix of terra firme forests (Figure 2.1).

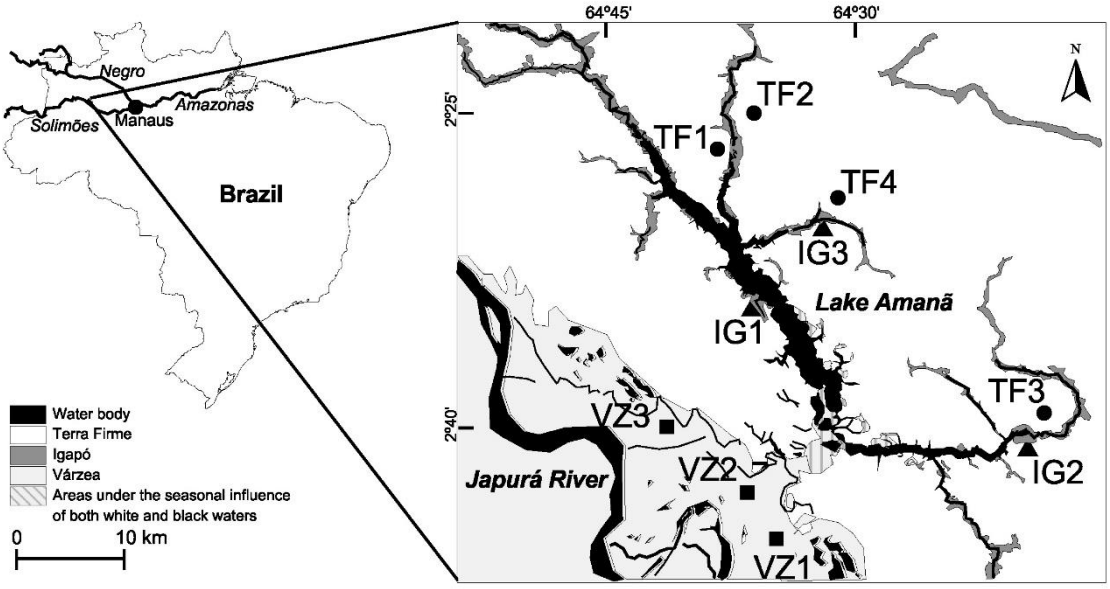

Figure 2.1 Location of sampling sites (TF – terra firme, VZ – várzea, IG – igapó). The southern part of the lake usually has black water but can temporarily receive white waters. Distribution of habitats based on cartography by the Mamirauá Sustainable Development Institute and satellite imagery.

Lake Amanã (2º37'S, 64º37'W) is a mostly black-water lake fed predominantly by streams draining catchments dominated by terra firme. Igapó occurs mainly along the streams and margins of Lake Amanã, while most várzea is located in the floodplain of River Japurá. Human population density is very low and only ca 4000 people live

inside the reserve. The area receives about 2500 mm of annual precipitation, mostly during the high-water season, from January to June. The low-water season usually extends from July to December. Water levels vary up to 10 meters between the two seasons. Flowering and fruiting peaks usually occur in the low and high-water seasons, respectively (Ayres 1993). The dominant trees belong to the families Sapotaceae, Lecythidaceae, Euphorbiaceae, Myristicaceae and Leguminosae (Ayres 1993; Ramos Pereira *et al.* 2010a). Canopy height is usually between 15 and 35 m, with emergent trees reaching at least 50 m (Ayres 1993).

Bat and diet sampling ´

Bats were captured between April and June 2007, when várzea and igapó forests are flooded, and between October and December 2007, when all forests are dry. We sampled ten stations, of which four were in terra firme, three in várzea, and three in igapó. Each station was sampled during four consecutive nights using three 3 x 12 m mist nets set at canopy level (17-35 m high) and ten 12 x 3 m nets at ground level. During the high-water season ground mist nets were set above water in both várzea and igapó, but canopy nets were not used in these habitats because of logistical difficulties. The nets remained open between 18:00 and 24:00 h, because most bats tend to be captured in the early evening, and were checked every 20 minutes. Each captured bat was sexed, weighed, and identified using identification keys (Lim and Engstrom, 2001; Sampaio, Kalko and Wilson, personal communication). We made temporary marks on the patagium of bats using a pen marker, to allow recognition of recaptures over the same four day sampling period. However, there were only four recaptures and these were not included in the analyses.

The diet of the captured bats was studied by analyzing items found in feces. We kept each bat in a separate cotton bag for about 30 minutes to collect feces. These were then air dried and, using a stereo microscope, their contents were separated in five categories: seeds, fruit pulp, arthropods, pollen and flower parts, and undetermined. Seeds were identified using a reference collection from the site. It is worth noting that the medium- and large-sized frugivorous bats included in our analysis may consume forest fruits that were not recorded because the seeds are not ingested (*e. g.* Sapotaceae, Humiriaceae, Chrysobalanaceae, Araceae - see Lobova *et al.*, 2009).

Fruit availability

In each of the ten sampling stations we used two 1km long fruit availability transects. One of the transects was located in the forest interior, partly coinciding with the location of the mist-nets, while the other was located along the nearest river bank. We identified all trees with canopies intersecting the transect line. Using binoculars and with the help of a local field assistant with extensive experience in the collection of plant phenology data, we determined which of those trees had ripe fruits. We also identified all fruiting shrubs within 2 meters of the transect line. The results of this method are approximate but since we used the same methodology and observer at all sites, we consider them suitable for the comparisons that we made. We estimated fruiting tree abundance in both high-water and low-water seasons, coinciding with the periods of bat captures. We present results on the availability of the main fruit genera consumed by the different bat foraging guilds. We also estimated the numbers of ripe fruits on trees and shrubs. However, the huge variability in numbers of fruits, especially because of figs, rendered these data unsuitable for the comparisons.

Data Analysis

The importance of each fruit type in the bats' diet was quantified using the frequency of occurrence, *i. e.* the percentage of samples containing seeds of that fruit. Diet data were pooled by plant genus because we were often unable to identify the fruit remains to the species level. Only bat species with a minimum of eight diet samples containing seeds were included in the diet data matrix. This matrix was explored for the presence of feeding guilds by visually searching for clusters of species and fruits in the plot of the first two axes of a Correspondence Analysis implemented with PAST (version 1.90, Hammer *et al.*, 2001).

Differences in fruit production between the three forest types and between the forest matrix and river bank vegetation were evaluated using the numbers of fruiting plants. Prior to statistical analyses the variables were log-transformed to approximate normality and reduce the influence of extreme values (Zuur *et al.*, 2007). We used two-way repeated measures ANOVA to assess differences between the three forest types and transect location (forest matrix vs. river bank). This method accounts for the lack of independence (*i. e.* pseudoreplication, Hurlbert, 1984) caused by estimating fruit availability on the same transects during both seasons. ANOVA tests and Tukey HSD were done in R (version 2.10.1). The threshold for statistical significance was $P \leqslant 0.05$, although we also report and discuss near significant probability values, *i. e.* $P<0.1$.

2.4 Results

During the 80 nights of mist-netting we captured 1242 bats of 60 species, and collected a total of 599 diet samples from 32 bat species (305 in the high-water season and 294 in the low-water). Frugivore species represented 69.5% of the total of diet samples, and the best represented species were the frugivorous *Carollia perspicillata* and the omnivorous *Phyllostomus elongatus* (Table 2.1).

Diet and Feeding guild identification

Ten frugivorous bat species were included in the diet matrix, using a total of 199 diet samples for the high-water season and 171 samples for the low-water (Table 2.1).

Table 2.1 Numbers of bat captures and diet samples, and frequency of food items on fecal samples of bat species captured more than 15 times.

		Diet samples				Food items				
Species	Captures	High-water	Low-water	Ground level	Canopy level	Seeds	Insects	Fruit pulp	Nectar, pollen	Und.[b]
Carollia perspicillata[a]	253	114	69	161	22	132	31	29	-	9
Artibeus planirostris[a]	193	26	49	59	16	59	2	14	-	2
Phyllostomus elongatus	135	44	60	-	-	4	98	14	-	-
Glossophaga soricina	65	6	12	-	-	1	6	7	7	-
Rhinophylla pumilio[a]	46	14	9	13	6	11	2	6	-	8
Sturnira tildae[a]	26	11	4	12	2	13	2	3	-	3
Artibeus lituratus[a]	19	6	4	6	4	8	1	3	-	-
Artibeus obscurus[a]	70	9	6	12	3	9	1	6	-	1
Carollia castanea[a]	19	10	6	14	2	14	2	3	-	2
Lophostoma sylvicolum	24	8	9	-	-	-	15	2	-	-
Mesophylla macconelli	42	6	3	-	-	4	-	5	-	2
Phyllostomus hastatus	16	10	4	-	-	5	9	4	-	1
Plathyrrhinus helleri[a]	41	4	9	6	7	12	2	-	-	-
Uroderma bilobatum[a]	20	4	8	5	7	11	1	1	-	-
Vampyriscus brocki[a]	16	1	7	1	7	8	-	-	-	1
Tonatia saurophila	25	7	6	-	-	5	9	3	-	-

[a] Bat species with eight or more fecal samples with seeds that were selected for further analysis (see Methods). [b] Undetermined.

The correspondence analysis of the diet matrix revealed a clear structure relating frugivorous bats and the plant genera they consume. Bats that fed on fruits in the canopy were clearly separated from those that usually forage in the forest understorey along the first axis (Figure 2.2), which had an eigenvalue of 0.68, indicating a good separation of the species along that axis. The “canopy guild”, included bats that consumed almost exclusively *Ficus* and *Cecropia* fruits - *Platyrrhinus*

helleri, *Vampyriscus brocki*, *Uroderma bilobatum*, *Artibeus planirostris*, *Artibeus lituratus* and *Artibeus obscurus*. The “understorey guild”, included *Rhinophylla pumilio*, *Carollia castanea, Sturnira tildae*, and *C. perspicillata*, which fed mostly on understorey plants, in particular of the genera *Vismia*, *Piper*, and *Philodendron*. Of the 745 captured frugivorous bats, 703 belonged to the ten species that we were able to assign to one of the two feeding guilds (359 of the canopy guild and 344 of the understory guild).

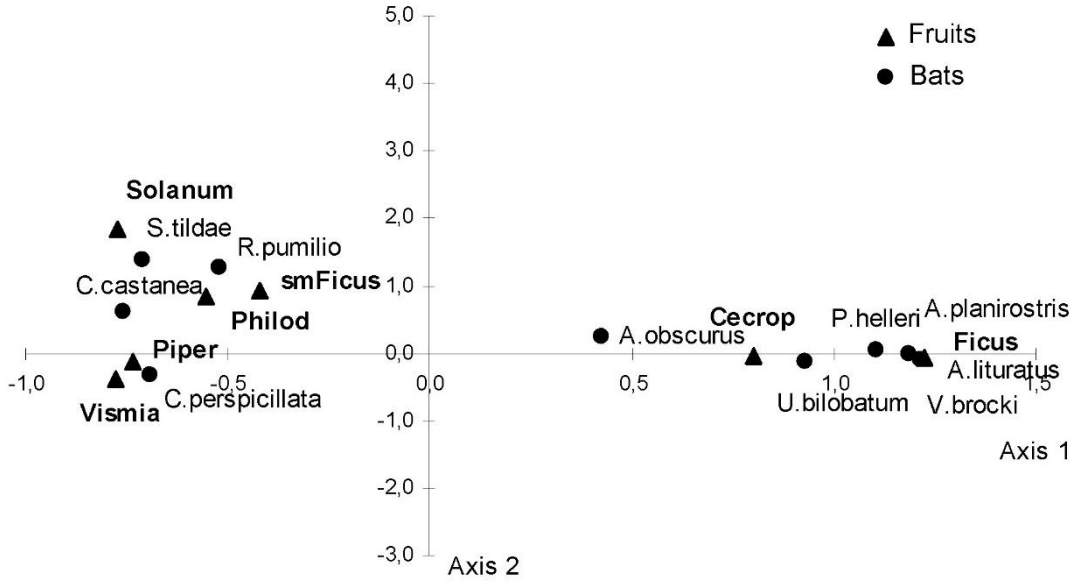

Figure 2.2 Correspondence Analysis of the diet matrix with bat species and fruits consumed (eigenvalues: Axis 1= 0.687 and Axis 2= 0.201). Fruit labels in bold: Solanum - *Solanum* spp.; smFicus - small seeded *Ficus* species; Philod - *Philodendron* spp.; Piper – *Piper* spp.

To assess the contribution of each fruit genus to the diet of the two feeding guilds in our study region, we graphed the combined data for all bat species included in each guild. We identified at least six species (or morphotypes) of *Piper* (*Piper alatabacum*, *Piper arboreum*, *P. dilatatum*, *Piper sp.* 1*, Piper sp*. 2 and *Piper sp*. 3), three of *Cecropia* (*Cecropia sciadophylla*, *C. membranacea* and *C. sp.* 1), six species of figs (*Ficus maxima*, *F. nymphaeolia*, *Ficus sp.* 1, *Ficus sp.* 2*, Ficus sp.* 3 and *Ficus sp.* 4), three species of *Vismia* (*Vismia cayennensis*, *Vismia sp.* 1 and *Vismia sp.* 2) and four morphotypes of *Philodendron*. The diet of the canopy guild was mainly

composed of *Ficus* and *Cecropia* fruits (Figure 2.3), whereas that of the understorey guild was dominated by fruits of *Vismia* trees and *Piper* shrubs (Figure 2.3).

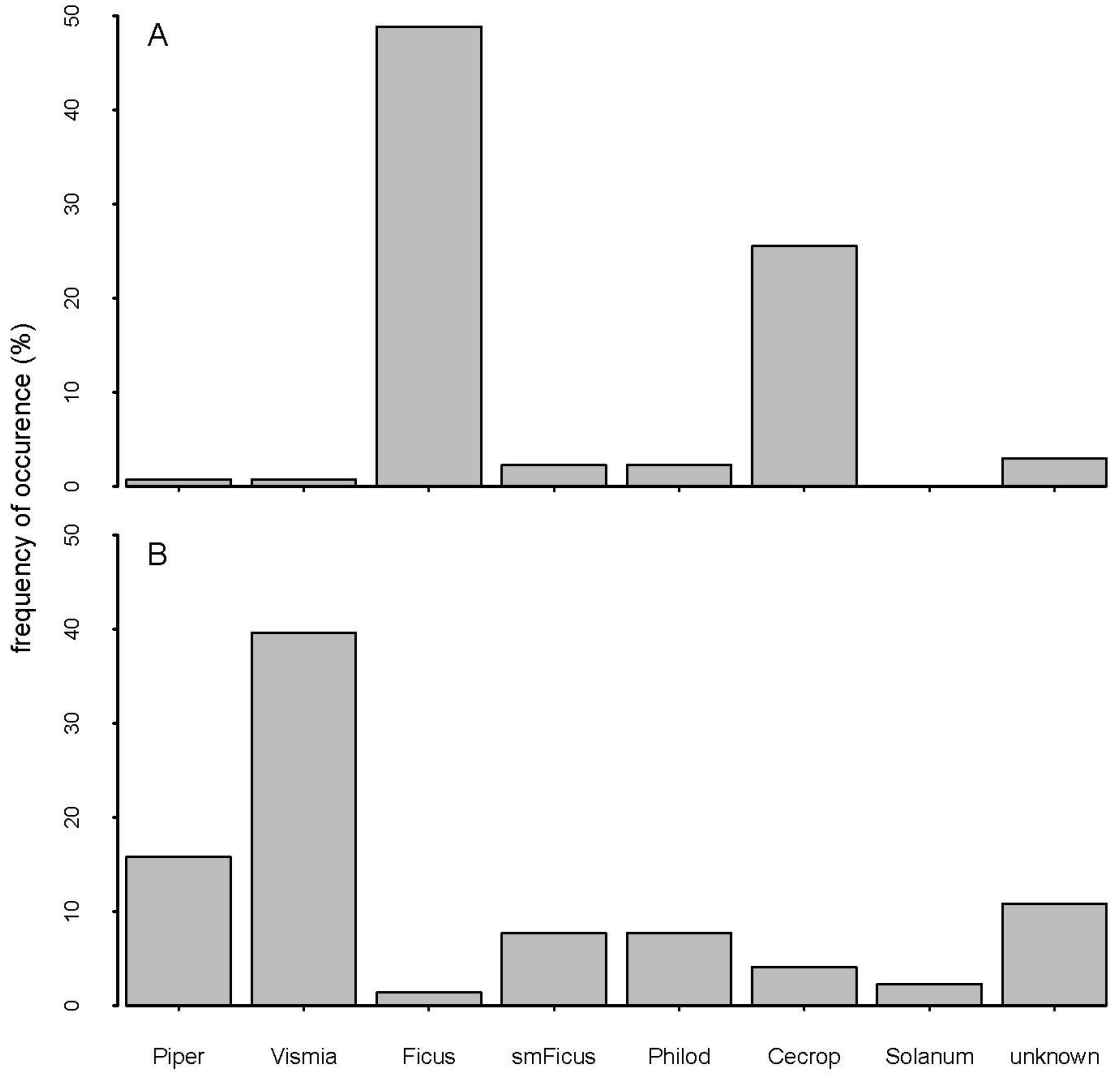

Figure 2.3 Diets of canopy (A) and understory (B) guild bats, expressed as frequency of occurrence of seeds in samples. Data of low and high-water seasons are pooled. Fruit labels as in Figure 2.2.

Bats of each guild consumed mainly fruits of their corresponding height stratum, irrespectively of the forest type (Figure 2.4); canopy bats consumed mainly fruits available in the forest canopy in terra firme, igapó and várzea forests, and understorey bats consumed mainly fruits only available in the understorey, even in the flooded forests.

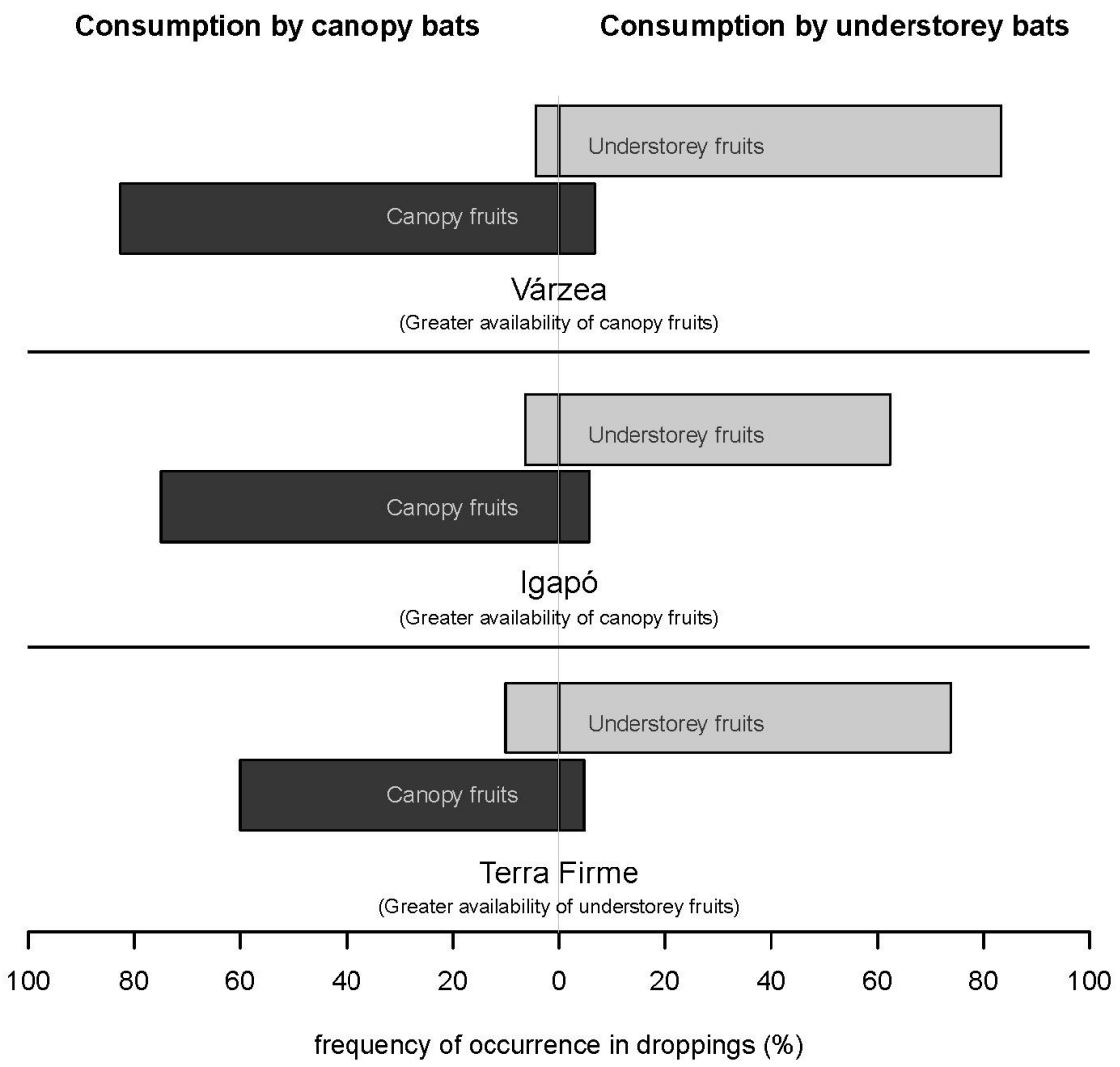

Figure 2.4 Strength of the association of bats to their feeding guild. Bats continue selecting fruits typical of their guild even in habitats where they are scarce, instead of switching to the types of fruit that exist in greater abundance in each habitat, demonstrating a strong association with their feeding guild.

Fruit availability - Differences between forest types

We assessed the availability of food resources for the canopy bat guild using the fruiting trees of *Ficus* and *Cecropia*, which dominated the diet of this guild. Fig trees bearing fruits were more abundant in várzea than in igapó and terra firme ($F_{2,14}$=6.35,

P=0.01). *Cecropia* fruiting trees appeared to be more abundant in várzea, although differences from the other two types of forests were only nearly significant ($F_{2,14}$=2.97, P=0.08).

Core fruits consumed by the understorey foraging guild, including the highly consumed *Vismia* and *Piper* genera, were in general very scarce; in the fruit availability transects we only recorded fruiting *Vismia* and *Piper* plants in terra firme, and always in relatively low numbers (Figure 2.5).

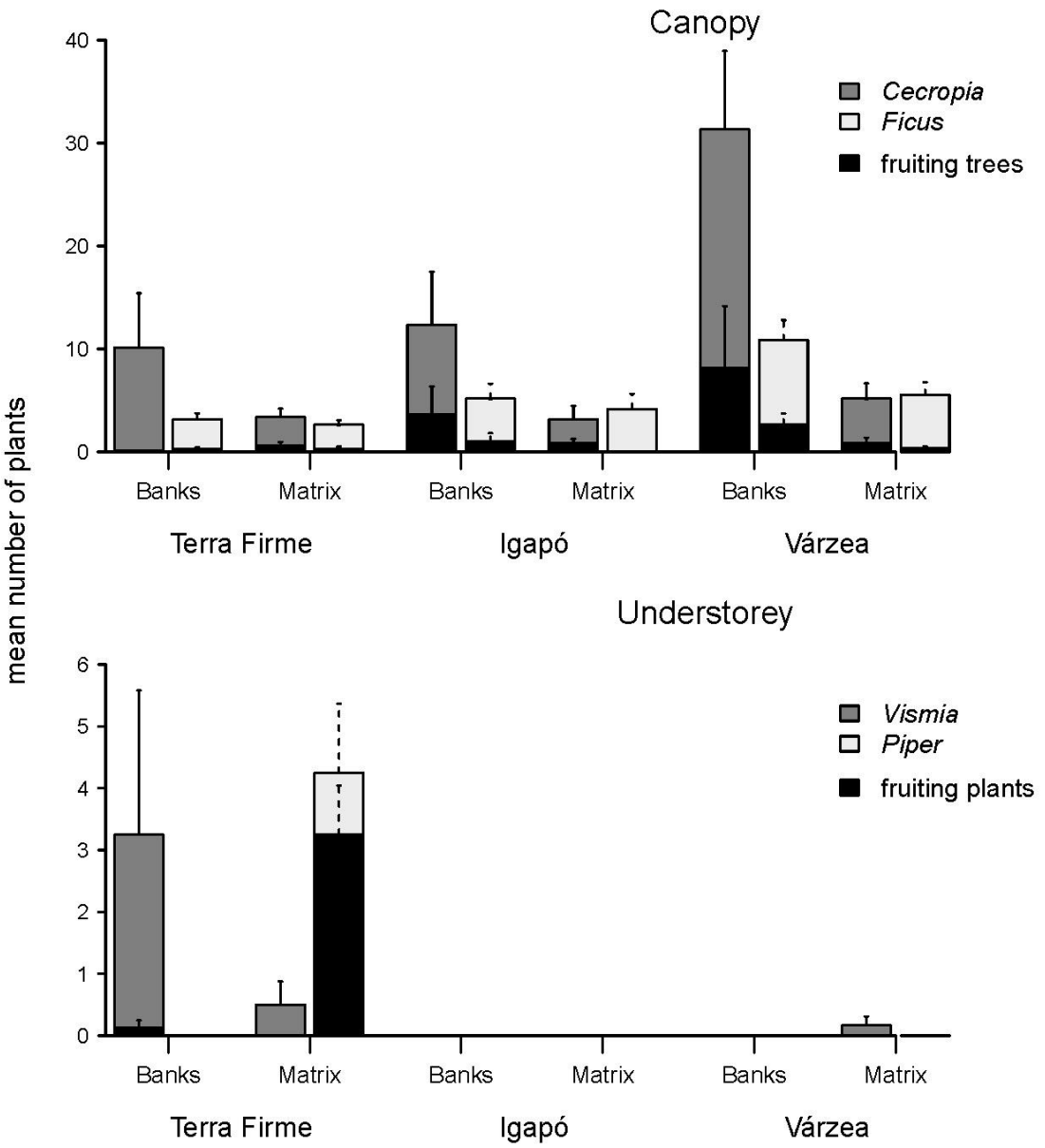

Figure 2.5 Number of plants and of plants with fruits of the genera most consumed by the two bat guilds in the three types of forest, contrasting river banks with forest matrix. Lines represent standard error of the mean.

Fruit availability - Differences between riverbank and matrix vegetation

We found similar numbers of *Ficus* trees in transects of river bank vegetation and forest interior, but individuals bearing fruits were more numerous in river banks than in the forest matrix ($F_{1,14}$=5.29, P=0.04) (Figure 2.5). *Cecropia* trees were also more numerous in river bank vegetation than in the forest matrix, but differences were only nearly significant ($F_{1,14}$=3.65, P=0.06). The number of fruiting Cecropia was several times higher in river bank vegetation than in the forest interior of igapó and várzea (Figure 2.5), but presumably due to high variability in the data the differences were not statistically significant ($F_{1,14}$=0.74, P=0.40).

Understorey fruit availability also varied between river bank vegetation and the forest matrix (Figure 2.5). Vismia fruiting shrubs were only recorded in river banks of terra firme, and even there at very low densities. In contrast, Piper fruiting plants were only recorded in the forest matrix, so the overall river bank / matrix contrast in understory fruit availability was not as marked as that observed for the canopy guild.

2.5 Discussion

Feeding Guild Structure

Several studies conducted in terra firme forests have identified two guilds of frugivorous bats, the canopy and understory guilds (Bonaccorso, 1979; Palmeirim *et al.*, 1989; Bernard, 2001; Delaval *et al.*, 2005; Rex *et al.*, 2008). We were able to confirm for the first time the presence of both guilds in two seasonally flooded forest types, várzea and igapó, in spite of the structural differences between these forests and terra firme. Flooded forests usually have lower canopy cover and sparser understorey than terra firme (Borges and Carvalhães, 2000; Haugaasen and Peres, 2006).

Our results confirm that *Ficus* and *Cecropia* fruits are key resources for the majority of canopy frugivores. The group that dominates this guild, the Stenodermatini, are considered *Ficus* specialists (*e. g.* Bonaccorso, 1979; Giannini and Kalko, 2004), although as we observed, they can also consume large numbers of *Cecropia* fruits (Lobova *et al.*, 2009; Teixeira *et al.*, 2009). In the study region, the high abundance of *Cecropia* and its long fruiting period (Myster, 2009; JTM and MJP, personal observation), suggests that these plants provide a reliable food supply throughout most of the year.

One of the bat species included in this guild, *Vampyriscus brocki*, is a poorly known Amazonian endemic (Marinho-Filho and Sazima, 1998). Our results indicate that this rare species feeds mainly on *Ficus* and should therefore be included in the

canopy frugivore guild, as suggested by Bernard (2002). In fact, most of the few known captures of this species took place in the canopy (Bernard, 2001; Barnett *et al.*, 2006; Ramos Pereira *et al.*, 2010b).

Vismia fruits were most consumed by understorey frugivores, particularly by *C. perspicillata*. Several other studies have also found *Vismia* dominating the diet of understorey bats (Gorchov *et al.*, 1995; Bernard, 2002; Sampaio *et al.*, 2003; Lobova *et al.*, 2009), but in many regions *Piper* is their main food resource (Fleming, 1988; Palmeirim *et al.*, 1989; Thies and Kalko, 2004; Aguiar and Marinho-Filho, 2007). Presumably, understorey bats switch between *Vismia* and *Piper* in response to the local availability of these plants. This may explain the dominance of *Vismia* in our data; *Piper* is quite scarce in the study area, possibly because its forests are nearly pristine, and mature forests tend to have a low abundance of *Piper* (Fleming, 2004).

In spite of the prevalence of *Vismia*, none of the understory frugivores is entirely dependent on fruits of this genus. *R. pumilio*, *C. castanea* and *S. tildae* frequently also consumed *Philodendron*, *Piper* and *Solanum*, respectively. *Philodendron* fruits actually constituted the main dietary item of *R. pumilio*, corroborating their reported preference for epiphyte infructescences (Henry *et al.*, 2007). *C. castanea* seems to be more dependent on *Piper* fruits than *C. perspicillata*, as reported for other regions (Palmeirim *et al.*, 1989; Thies and Kalko, 2004). *S. tildae* consumed mostly fruits of *Solanum* spp., in spite of the apparent scarcity of these plants in the study region. Henry *et al.* (2007) also noted their paucity in undisturbed forests in French Guyana and suggest that they are usually scarce in this habitat.

Strength of association of frugivorous bats to their feeding guild

The consistent structuring of Neotropical frugivorous bat assemblages in two major feeding guilds, canopy and understory, suggests that species have adaptations that make them specialists in the exploitation of either forest strata. But how strong is the association of these bats to their feeding guild? Where the foods typical of their guild are scarce will they switch to those of the other guild? Our study area is particularly suited to answer this question, because it encompasses forest types in which the availability of the typical foods of the two guilds are very different. If the level of specialization of canopy bats to fruits of that stratum is low then we predict that in terra firme, where *Ficus* and *Cecropia* are scarce, they would switch to understorey fruits; conversely, if their level of specialization is high, we predict that their diet would remain the same, and their abundance would decline when canopy fruits are scarce. Likewise, if understorey bats are highly specialized, then we can assume that they will not switch to canopy fruits in flooded habitats, where their preferred understorey fruits are less abundant.

Our diet data and the results of the correspondence analysis corroborate the predictions made under the scenario of specialization and strong association with one of the guilds - bats did not switch to fruits of the other guild, even where the typical foods of their own guild were scarce. In terra firme, where canopy fruits were least abundant, understory fruits were present in less than 10% of diet samples of canopy guild bats, indicating a strong attachment to their typical fruits. Likewise, in the flooded forest types, where understory fruits were very scarce, the proportion of canopy fruits in the samples of understory guild species was very low (5.7% in igapó and 6.7% in várzea). We conclude that there must be important constraints that limit bats to exploit

the fruits of a specific vertical stratum (*e. g.* flight or trophic morphology and/or sensory adaptations). This specialization may have resulted from an evolutionary process of niche partitioning to reduce competition among Neotropical frugivorous bats (DeLaval *et al.*, 2005). It is worth noting, however, that some canopy bats did eat a few understory fruits, and that several understory bats ate canopy fruits. In fact, the most abundant bat of the understorey guild, *C. perspicillata*, is known to have a particularly diverse diet that can include fruits of both *Cecropia* and *Ficus* (Bonaccorso, 1979; Lobova *et al.*, 2009), fruits that are typical of the canopy guild.

Influence of forest type on the availability of fruits for the canopy and understorey guilds

The main sources of food for the canopy guild, fruits of *Ficus* and *Cecropia*, were more abundant in várzea, a nutrient-rich habitat, than in igapó and terra firme, both nutrient-poor forests. In fact, *Ficus* trees tend to be more abundant in rich soils (Gentry, 1990), and *Cecropia* is known to form large monospecific stands in várzea areas (Parolin *et al.*, 2002). Consequently, the greater availability of canopy fruits recorded in várzea probably results from the high nutrient content of the water that inundates and fertilizes these forests (Furch, 1997). Data in Haugaasen and Peres (2007) also suggest a higher productivity of fruits in várzea than in igapó and terra firme forests. The greater availability of *Ficus* and *Cecropia* fruits in várzea may explain why its bat biomass, dominated by canopy guild species, was twice that of the other two nutrient-poor types of forest (Ramos Pereira *et al.*, 2009). Sampaio *et al.* (2003) also explained the low abundance of canopy bats in terra firme forests near Manaus as resulting from the low availability of *Ficus* trees, possibly a consequence of nutrient poor soils (Gentry, 1990).

Contrasting with most canopy guild species, the fig-eater *V. brocki* was more abundant in terra firme forests (Ramos Pereira *et al.*, 2009). This may be explained by its roosting preferences; presumably it roosts under leaves in the forest understorey, as described for related species (Kunz *et al.*, 1994), and such roosts are likely to be rarer in the comparatively sparse understory of flooded forests.

Although the diet of the understory bat guild was more diverse overall than that of the canopy guild, these bats were also heavily dependent on just two plant genera, *Piper* and *Vismia*. It is thus somewhat surprising that fruiting plants of both genera seem to be rare in terra firme, and nearly absent in flooded forests. The absence of *Piper* shrubs in flooded forests is probably a consequence of an inability to resist the annual flooding; most species of this genus are generally found on well-drained soils (Marquis, 2004). In the case of terra firme the low observed abundance may be due to the fact that the sampled sites are virtually dominated by closed undisturbed forest. In similar forests Bonaccorso *et al.* (2007) also found very few *Piper* plants that were small in stature and bearing almost no fruit, and Fleming (2004) reports that *Piper* tends to become scarcer with increasing maturity of the forest. Moreover, Pipers may have non-uniform distributions (Bernard and Fenton, 2003), because many are pioneer species, occurring mostly in gaps and along edges (Lobova *et al.*, 2009). The scarcity of fruiting *Vismia* may also be explained by their marked pioneer character (Lobova *et al.*, 2009). In most of the study region habitats disturbed by human activities are quite rare and localized, so pioneer plants are probably only present in naturally disturbed areas. As in some species of *Piper*, this dependency on habitat disturbances may also result in a patchy distribution, which makes their abundance difficult to estimate. This limitation should be considered when interpreting our estimates of understory fruit abundance.

In spite of the apparent scarcity and patchiness of *Vismia* and *Piper* fruits, they remain important in the diet of some species of the understory guild, implying that

foraging bats travel to patches with particular characteristics to feed. In the case of flooded forests, understory bats presumably travel to elevated areas, islands or adjacent terra firme, which should have more fruit-producing shrubs than the surrounding inundated areas. A similar foraging strategy is followed by several terrestrial mammal species inhabiting Amazonian flooded forests (Bodmer, 1990).

In conclusion, we found that the availability of food for frugivorous bats varies among the three types of forest, a situation already described for other vertebrates in similar Amazonian forest mosaics (Haugaasen and Peres, 2007). The main fruits consumed by canopy bats are more abundant in várzea than in both igapó and terra firme, and the abundance of canopy bats reflects this difference. The situation is different for understory bats, as their key fruits were scarce in the three habitats, although less so in terra firme.

The importance of river banks

Our results suggest that river bank vegetation is very important for bats foraging in lowland Amazonian forests because these areas have more fruits available for bats to eat. However, the differences in occurrence and abundance of fruits between river banks and forest interior do not have the same implications for understorey and canopy bats.

Canopy bats had far more food available, particularly fruits of *Ficus* and *Cecropia*, along river banks in the two types of flooded forests. Such pioneer and early successional trees are usually abundant in the new soils that result from the accumulation of sediments along the inner banks of river meanders (Parolin *et al.*, 2002; Muscarella and Fleming, 2007; Schöngart *et al.*, 2007). This occurs in igapó and

várzea but is more accentuated in the latter because white water has higher sediment loads (Furch, 1997).

In our study area terra firme was an exception to this trend for greater abundance of *Ficus* and *Cecropia* along the river banks. This is due to the fact that this type of forest only occurred on the high outer curve of the meanders. The remaining river bank area was occupied by igapó. These high banks, which are retreating due to river erosion, are steep and occupied by mature forest to the edge, so there is very little space for the establishment of pioneer and early successional trees. The situation may be different in Amazonian regions where igapó does not dominate the river bank areas; the beaches that build up in the inner banks are then occupied by the very early stages of the terra firme forest, which usually have a great availability of pioneer *Cecropia* spp. trees (Kalliola *et al.*, 1991).

Of the two fruits most consumed by understorey bats, *Vismia* and *Piper*, the first was only found along river banks but the second was mostly present, albeit in low numbers, in the forest matrix. Different resistance to flooding probably explains the distinct spatial distribution of the two genera. In fact, *Vismia* shrubs and trees are mainly found near creeks and rivers (van Roosmalen, 1985; Ferreira, 2000), while *Piper* species prefer well-drained soils (Marquis, 2004). Consequently, in terra firme the higher abundance of *Vismia* on the edges is balanced by a greater abundance of *Piper* in the forest interior. This makes the contrast in food availability between bank and forest matrix less marked for the understory guild than for the canopy guild.

The main reason why bat fruits are so abundant in river banks is because they are often pioneer and early successional plants. In pristine Amazon habitats such plants occur mostly in the areas that are disturbed by river dynamics or light gaps due to tree falls, with the former covering much greater areas. Salo *et al.* (1986) estimated that 12% of the Peruvian lowland Amazon is in successional stages along rivers,

whereas the proportion of forest in early regeneration due to tree falls is typically 3-7% (*e. g.* Hartshorn, 1978). Moreover, river bank vegetation is predictable in space because it occurs in continuous strips (Salo *et al.*, 1986), so presumably bats find food along them more efficiently than in the dispersed tree fall gaps. In fact, it has been demonstrated that bats more readily find fruits along their flyways (Palmeirim and Etheridge, 1985) and they use tropical river systems as flyways (Fleming *et al.*, 1972; Delaval *et al.*, 2005; Medina *et al.*, 2007), taking advantage of the less cluttered air space (Meyer *et al.*, 2005).

Because frugivorous bats often fly along rivers and defecate in flight (Whittaker and Jones, 1994), seed dispersal may also reinforce the abundance of bat fruits in riverine vegetation; river banks may receive more seed rain of bat-consumed plants than matrix habitats. Fishes, which are known to be important dispersers of *Cecropia* (Kubiztki and Ziburski, 1994) and *Ficus* fruits (Banack *et al.*, 2002), may also contribute to the greater abundance of these plants on river banks. Other frugivores, including birds and mammals, may disperse seeds mostly along rivers, but to our knowledge this has not been studied.

The greater abundance of bat-consumed fruits in river bank vegetation makes this a potential keystone habitat within lowland Amazonia. Of particular importance is the greater abundance of *Ficus* spp., which are known to be a key resource not just for bats but also for other vertebrate groups (Shanahan *et al.*, 2001), such as primates (*e. g.* Peres, 1994) and birds (*e. g.* Shanahan and Compton, 2001). It would now be interesting to study if the nutritional content of fruits in river bank vegetation differs from those of the different types of forest matrix, as this may be relevant for the choice of foraging habitats by frugivores.

Our conclusion that bat fruits are more abundant along Amazonian river banks explains why some frugivorous bats occur in greater numbers in this habitat (Fleming

et al., 1972; Delaval *et al.*, 2005). Bat species that exploit these river bank resources tend to become more numerous, which has consequences for the structuring of bat communities. Delaval and Charles-Dominique (2006) demonstrated that edge effects on frugivorous bats are evident at least 3 kilometres away from the disturbed edges, presumably because bats can commute long distances to feed. It is thus likely that the abundant fruit resources of river banks influence the structure of frugivorous bat communities far into the forest matrix.

2.6 Acknowledgements

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Chapter 3

Patterns in the use of rainforest vertical space by Neotropical aerial insectivorous bats: all the action is up in the canopy

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3 Patterns in the use of rainforest vertical space by Neotropical aerial insectivorous bats: all the action is up in the canopy

3.1 Abstract

In tropical rainforests environmental conditions vary dramatically from the ground to the canopy, resulting in a marked stratification in the way vertical space is used by organisms, but research work is often limited to the understorey layer. Aerial insectivorous bats are a highly diverse group that plays key roles in the ecology of rainforests, but their use of vertical space remains elusive.

Using automatic ultrasound recording stations placed in the canopy, subcanopy and understorey we tested if bat activity and species diversity are vertically stratified, both in the forest interior and near the edges of water bodies. These patterns were tested separately for individual species, and for two functional groups - open space and edge space bats.

Insectivorous bat activity increased by roughly seven fold, and species diversity doubled, from the understorey to the canopy. Both edge space and open space bats were more active in the upper strata, but this tendency was much more accentuated in the latter. Myotis riparius was the only species with greater activity near the understorey. These patterns were altered at the edges of water bodies, where vertical stratification was much less marked.

The observed patterns are parsimoniously explained by constraints imposed by vegetation clutter that change across strata, which affect bat species differently. Only bats better adapted to closed spaces are usually capable of foraging within the understorey, whereas the majority of species can exploit the free spaces immediately

below the canopy; open space bats seem to concentrate their activity above the canopy. This importance of the inter strata open spaces for bat foraging highlights the need to preserve pristine stratified rainforests, as even selective logging usually disrupts vertical stratification. Moreover, the concentration of insectivorous bats at the upper strata of rainforests underlines the need to include canopy level sampling in ecological studies.

3.2 Introduction

Tropical rainforests are the most species rich terrestrial ecosystems and their vertical complexity is one of the main factors contributing to this richness (Moffett 2013, López-González *et al.* 2015). They usually associate a remarkable tree height to multiple layers of vegetation (Klinge *et al.* 1975, King *et al.* 2006) and a highly variable leaf density across those layers (Clark *et al.* 2008). In addition, there are dramatic vertical changes in environmental variables, such as temperature, humidity and light (Parker 1995). As a consequence of this vertical biotic and abiotic complexity, tropical rainforests have a great density of ecological niches, which represent opportunities for an enormous diversity of organisms.

Understanding the ecological consequences of vertical stratification for different groups of organisms is an important objective in rainforest ecology (Smith 1973) that has been the focus of multiple studies. These demonstrate that the use of space in rainforests has a marked vertical pattern in several groups of animals, including invertebrates (Basset *et al.* 2003), birds (Walther 2002), small mammals (Vieira and Monteiro-Filho 2003) and primates (Heymann *et al.* 2002).

Bats are the most diverse group of mammals in Neotropical rainforests, usually representing up to 60% of their diversity and biomass (Simmons and Voss 1998). Moreover, they fulfil many important ecological roles in these ecosystems acting as key seed dispersers and pollinators (Muscarella and Fleming 2007), limiting insect populations, and reducing insect herbivory (Kalka *et al.* 2008).

The powered flight of bats enables them to easily exploit different forest heights, and there are studies that show that in Neotropical rainforests species vary in the way

they use vertical space (Kalko and Handley 2001, Ramos Pereira *et al.* 2010, Rex *et al.* 2011). However, these studies were based on sampling using mist nets, which are far more efficient at capturing species of the family Phyllostomidae than those of other families of bats. Consequently, little is known about the use of rainforest vertical space by aerial insectivorous bats, which are mainly non-phyllostomid.

Aerial insectivorous bats mostly rely on echolocation for orientation and to detect and capture their arthropod-prey, typically on the wing. Their capacity to forage in more or less cluttered habitats varies, and this variation has been used to classify species in three broad groups: open space, background cluttered space, and highly-cluttered space foragers (Schnitzler and Kalko 2001). The two latter groups were recently renamed edge space and narrow space foragers, and divided into several sub-groups (Denzinger and Schnitzler 2013). The echolocation calls of most non-phyllostomid bats are relatively easy to record, and during the last few years there were substantial advances in the knowledge required for species identification of these calls in the Neotropics (Rydell *et al.* 2002, Jung *et al.* 2007, Williams-Guillén and Perfecto 2011, Barataud *et al.* 2013).

The development of instruments for automated ultrasound recording, which can be placed up in the forest canopy, is opening the possibility of studying the use of vertical space by aerial insectivorous bats, and this has been done with success in temperate forests (Hayes and Gruver 2000, Plank *et al.* 2012, Müller *et al.* 2013). However, no such studies have been done in tropical rainforests, so in spite of the vertical complexity of these ecosystems the stratification of their use by aerial insectivorous bats remains virtually unknown. The overall aim of our study was to contribute to fill this knowledge gap using a multi-strata acoustic sampling approach. Our specific objectives were to test and describe the patterns of vertical stratification in the use of space by aerial insectivorous bats in Neotropical rainforests. This was done for individual species and for two major functional groups of aerial insectivorous bats -

open space and edge space bats (Denzinger and Schnitzler 2013). The main discontinuities in the vertical structure of natural rainforests are usually the forest edges along streams and lakes (Salo *et al.* 1986), so we also studied how their presence influences the patterns of use of vertical space by bats. Finally, we discuss the observed patterns in the context of the existing knowledge about bat biology and rainforest vertical structure.

3.3 Materials and Methods

Study area

The study area is located in the Amanã Sustainable Development Reserve (2º37'S, 64º37'W, Amazonas, Brazil), and includes areas of three types of forest: terra firme (unflooded), várzea (seasonally flooded by nutrient rich water) and igapó (seasonally flooded by nutrient poor water) (Prance 1979, Ayres 1993). Canopy height varies between 15 and 35m, with emergent trees often reaching 50m (Ayres 1993). The region receives approximately 2500 mm of annual precipitation, mostly during the high-water season, from January to June. The low-water season is usually between July and December, when all forests dry up (Ayres, 1993).

Ultrasound recordings

To investigate the vertical stratification of bat activity in forests, acoustic sampling was conducted at a total of 10 sites (four in unflooded forests, three in várzea and three in igapó), between October and December 2007, when all forests are dry. Sampling sites coincided with those used in a mist-net survey of birds and bats (for details see Beja *et al.* 2009, Ramos Pereira *et al.* 2009). Acoustic sampling was also carried out at ten sites on nearby river or lake margins. Surveys at each site included two consecutive nights, starting at sunset and lasting up to 6 hours (average = 239 min+15 (SD)), thus encompassing the activity peak of aerial insectivorous bats in Neotropical forests (Estrada-Villegas *et al.* 2010). Sampling was interrupted during periods of heavy rain.

Three ultrasound recording units were mounted simultaneously at each sampling site: one in the canopy (average height = 24 m), one in the subcanopy (average = 17 m) and one near the ground (average = 2 m). Each recording unit combined a D240x bat detector (Pettersson Elektronic AB, Uppsala, Sweden; frequency range 10–120 kHz), and a digital MP3 recorder (iAudio U2, Cowon, South Korea; bit rate 128 kbps, sampling frequency 44.1 kHz). To increase the probability of recording passing bats the trigger level of the detector was set to low and the gain to high. Each time a bat passed, the detector made a 1.7s recording, and then played it back with a 10x time expansion to the MP3 unit. A slingshot and a pulley-rope system were used to suspend the recording units in the canopy and subcanopy, and the ground units were mounted on the trunk of a nearby tree. They were housed in protective plastic containers holding the bat detector pointing up at a 45º angle and towards areas free of vegetation clutter, to increase the likelihood of recording bats

(Weller and Zabel 2002). Prior to deployment in the field an ultrasound emitter (Pettersson Elektronic AB, Uppsala, Sweden) was used to adjust and equalize the trigger level of all detectors.

Species identification

Bat vocalizations were analysed using Avisoft-SASlab Pro (Version 4.52, Avisoft Bioacoustics, Berlin, Germany). Call and sequence spectrograms were generated with a 256 point Hamming window. Although all registered bat passes were counted, only sequences including at least three successive calls were identified, except in the case of species with easily recognized call structures (*e. g. Rhynchonycteris naso*). For each recording call shape, call alternation, and number and energy of harmonics were noted. Peak frequency, call duration, call interval and terminal frequency were also measured. Bat vocalizations were compared with those in a Neotropical bat call library maintained by E. Kalko and K. Jung, a local call library compiled from hand released bats during the project, and with published information (O'Farrell and Miller 1997, Ochoa *et al.* 2000, Siemers *et al.* 2001, Rydell *et al.* 2002, Jung *et al.* 2007, 2014, Barataud *et al.* 2013). We excluded from the analyses recordings with less than three well defined pulses, which corresponded to approximately 25% of the total bat passes (excluded bat passes: n = 1143 in forest sites; n = 2298 in riparian sites). Because of the small distance separating the canopy and subcanopy stations, bats flying between them could be recorded in both. Using the registers of the synchronized clocks of the stations we identified these double recordings (n=121) and assigned them to the station with the strongest signal.

Despite recent advances in the description of the echolocation calls of Neotropical bats (Jung *et al.* 2007, 2014) gaps persist, particularly for emballonurids and high flying molossids. Thus, some calls of these groups had to be identified to a species group or sonotype: Large *Eumops sp.* (all Eumops with peak frequency 16-21 kHz), *Cynomops sp.*, *Centronycteris sp.*, *Diclidurus* sonotype 1 (with a peak frequency about 36kHz), and *Saccopteryx* sonotype 1 (with peak frequency alternating between 45 and 48 kHz) (Supplementary material Appendix 1, Figure 3.7-Figure 3.11). In the case of 361 bat passes we considered that the information available was insufficient to allow identification, so they were not included in the analyses.

Data analysis

We standardized bat activity by calculating the number of bat passes per hour for each height strata (canopy, subcanopy and ground) and recording night. We used Linear Mixed Models to examine the differences in bat activity between height strata for each species and species groups, with 'site' specified as random factor, and assessed significance with likelihood ratio tests (Zuur *et al.* 2009). All analyses were performed using the software R (version 2.15.0) and R package 'lme4'.

The number of bat passes is an activity index rather than a count of the number of individuals occurring in the area and may be only correlated to species abundance, thus we estimated species richness for each stratum and species group based on incidence data (*i. e.*, presence/ absence). We compared species richness between strata using sample based rarefaction curves with 95% confidence intervals, obtained by 1000 randomizations on EstimateS (Version 9, R. K. Colwell,

http://purl.oclc.org/estimates). Species with less than 10 passes in each habitat (forest interior and water margins) were excluded from activity analyses, but were used to generate the rarefaction curves. We assigned species to foraging guilds using as a reference the classification in Kalko *et al.* (2008).

3.4 Results

During the 20 nights of sampling in forest interior locations with the three recording stations, we registered 4191 passes belonging to 30 species and sonotypes of aerial insectivorous bats. The number of passes in the forest interior increased markedly with height; the stations set up at ground level recorded only 366 bat passes, whereas those set up at subcanopy and canopy levels recorded 1175 passes and 2650 passes, respectively. Virtually all individuals recorded belonged to species of the open space and edge space aerial foraging guilds.

The activity of aerial insectivores at river and lake margins was much greater than in the forest matrix; we recorded a total of 9155 bat passes belonging to 34 species and sonotypes, during 20 nights of sampling in these edge habitats. In contrast with the observations made in the forest interior, bat activity here was concentrated in the lower strata, close to the water surface; the lowest stations recorded a total of 4837 passes, against 2491 in the subcanopy and 1827 passes at the canopy level.

Almost all species recorded belonged to the families Emballonuridae, Molossidae and Vespertilionidae. The most recorded species were *Saccopteryx bilineata* in forest interior sites and *Eptesicus brasiliensis* on river bank sites.

Vertical stratification of activity of individual species

Almost all bat species clearly concentrated their activity in the upper strata of the forest, and were seldom registered by the lowest recording stations (Figure 3.1). Eleven species had significantly higher activity in the forest canopy and subcanopy compared to the ground level (Figure 3.1; Table 3.1). The same tendency was observed for three additional species, but did not reach statistical significance. *Myotis riparius* was the only species that was registered more often in the understorey than in the upper strata ($\chi^2 = 6.35$, DF= 2, $p = 0.041$).

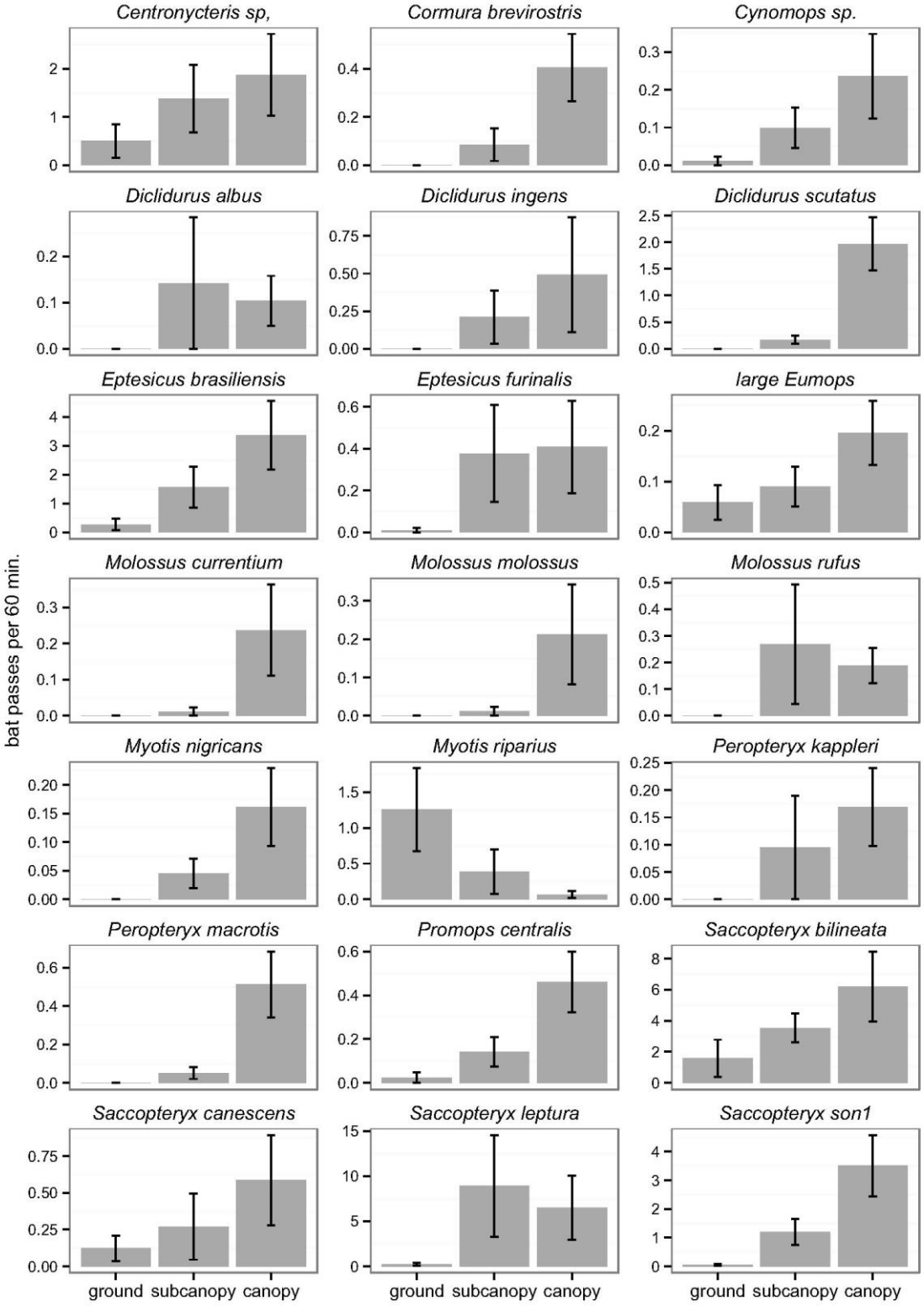

Figure 3.1 Activity of aerial insectivorous bat species at three height strata in the forest. Vertical lines represent one SE.

Table 3.1 Total bat passes in the three strata for species with more than 9 bat passes in forest or riparian sites. p – significance results of linear mixed models testing for differences among height strata obtained using likelihood ratio tests: * p<0.05; ** p<0.01; *** p<0.001.

	Forest				Riparian			
Species	Canopy	Subcanopy	Ground	p	Canopy	Subcanopy	Ground	p
Edge space								
Saccopteryx bilineata	327	186	54	0.017	89	97	187	0.269
Saccopteryx canescens	30	5	7	0.193	37	5	74	0.135
Saccopteryx leptura	318	158	9	0.097	60	36	85	0.238
Saccopteryx sonotype 1	272	82	3	<0.00	90	148	35	0.198
Cormura brevirostris	38	3	0	0.003	18	21	28	0.749
Centronycteris sp.	142	124	48	0.168	5	62	1	0.177
Eptesicus brasiliensis	149	64	10	0.015	144	186	843	0.062
Eptesicus furinalis	17	10	1	0.214	13	15	14	0.326
Myotis albescens	3	2	0	-	4	0	40	0.040*
Myotis nigricans	9	4	0	0.032	12	10	824	0.015*
Myotis riparius	4	41	101	0.041	11	22	11	0.293
Lasiurus ega	2	1	0	-	11	11	31	0.530
Lasiurus blossevilli	2	0	0	-	7	7	23	0.424
Rhynchonycteris naso	2	0	1	-	29	2	135	0.045*·
Open space								
Cynomops sp.	17	8	1	0.023	7	6	14	0.122
Large Eumops sp.	14	8	5	0.041	28	10	33	0.288
Promops centralis	31	9	2	0.004	70	32	45	0.984
Molossus currentium	11	1	0	0.051	31	5	15	0.275
Molossus molossus	13	1	0	0.105	63	81	50	0.520
Molossus rufus	15	5	0	0.323	23	9	13	0.828
Diclidurus scutatus	138	13	0	<0.00	184	107	317	0.095
Diclidurus albus	9	12	0	0.532	4	3	16	0.027*
Diclidurus ingens	28	18	0	0.393	18	10	67	0.182
Peropteryx kappleri	9	8	0	0.237	7	18	10	0.501
Peropteryx trinitatis	0	0	0	-	0	10	1	-
Peropteryx macrotis	36	3	0	0.002	59	14	113	0.360
Total sampling time (min.)	4649	4506	4175		4936	4088	4143	

Vertical stratification in species diversity

Species diversity also decreased with height; diversity at ground level was about half of that observed near the canopy (Figure 3.2).

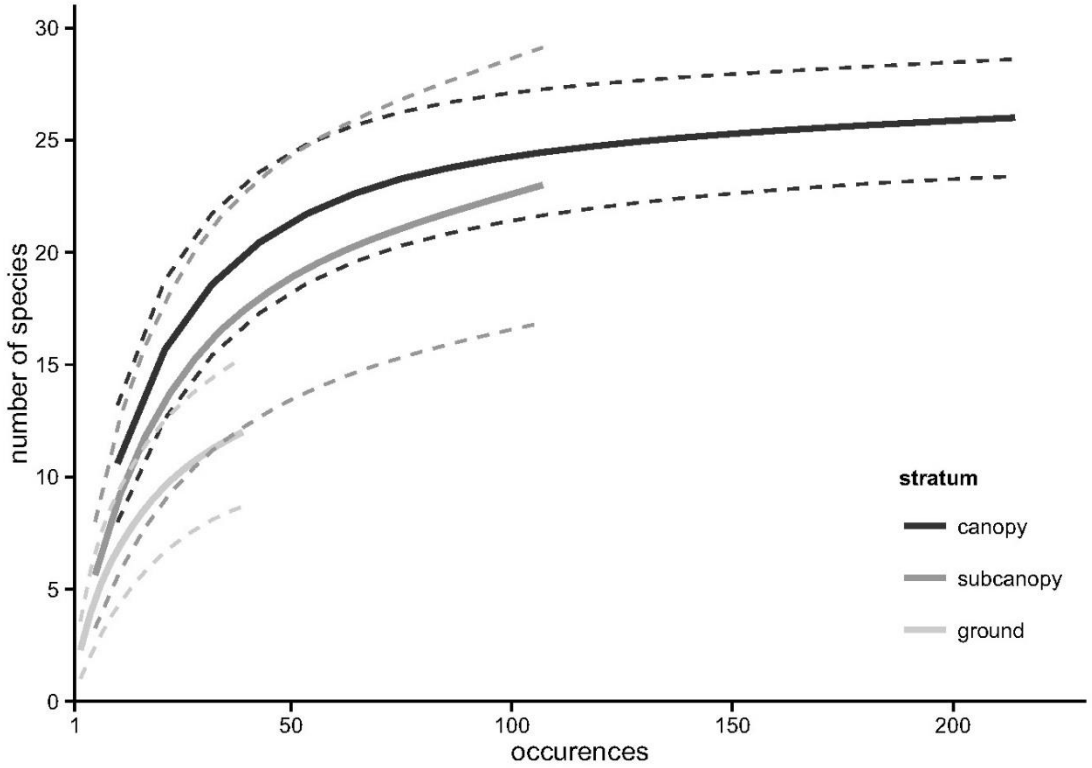

Figure 3.2 Species diversity in each of the three height strata. Solid lines are rarefaction curves with 95% CI (dashed lines).

Comparison between open space and edge space species

The activity of both edge space and open space bats increased with height (Figure 3.3), but they used vertical space differently. Edge space species had their activity concentrated in the two upper strata, subcanopy and canopy (χ^2 = 10.48, DF= 2, p = 0.005), but they were also regularly recorded near the ground. The activity of open space species was clearly greater at the canopy level (χ^2 = 20.55, DF= 2, p < 0.001), and only a small proportion of the recordings came from the subcanopy. They

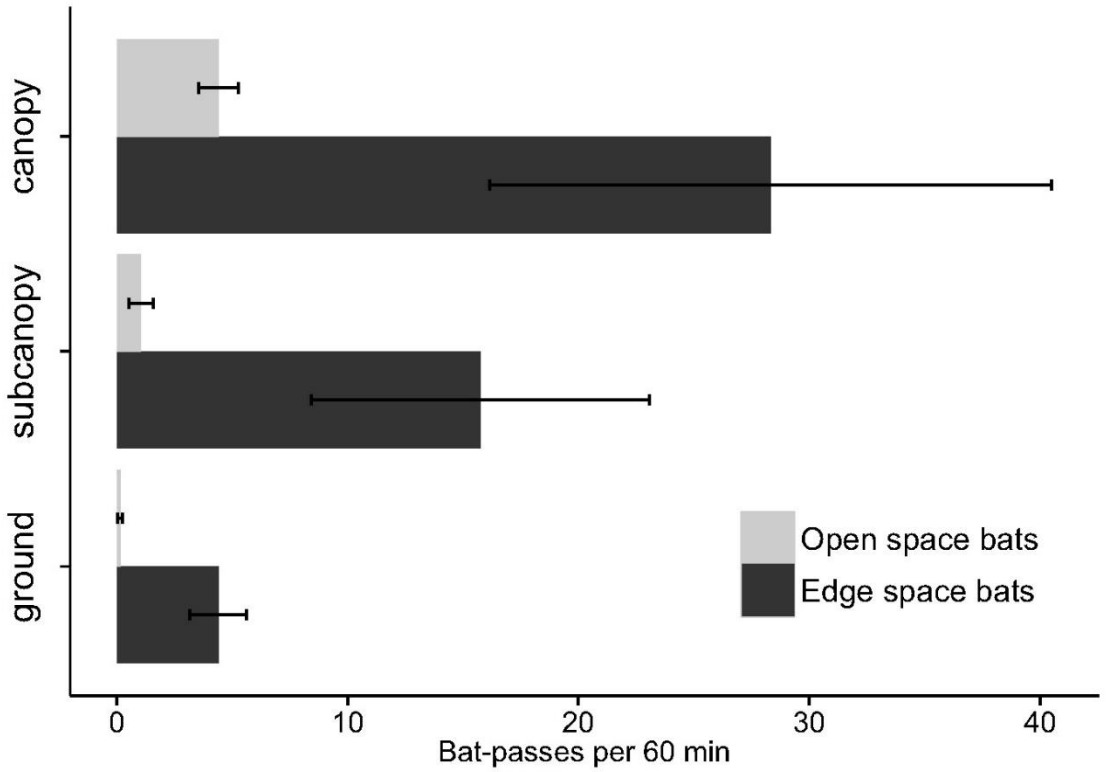

Figure 3.3 Activity of open space and edge space bats in the canopy, subcanopy and ground strata, at the forest interior sites. Lines represent one SE.

were very seldom registered at the stations placed near the ground.

To determine if species richness was greater near the canopy for both open space and edge space species, we generated separated species rarefaction curves for each of them (Fig. 4). Richness grew towards the canopy, even when analysing edge space species alone. This pattern was more accentuated in the case of the richness of open space bats, which was much greater at both upper strata than at ground level. In fact, we could not even generate a rarefaction curve for the latter because we only had 5 observations of 3 species of open space bats.

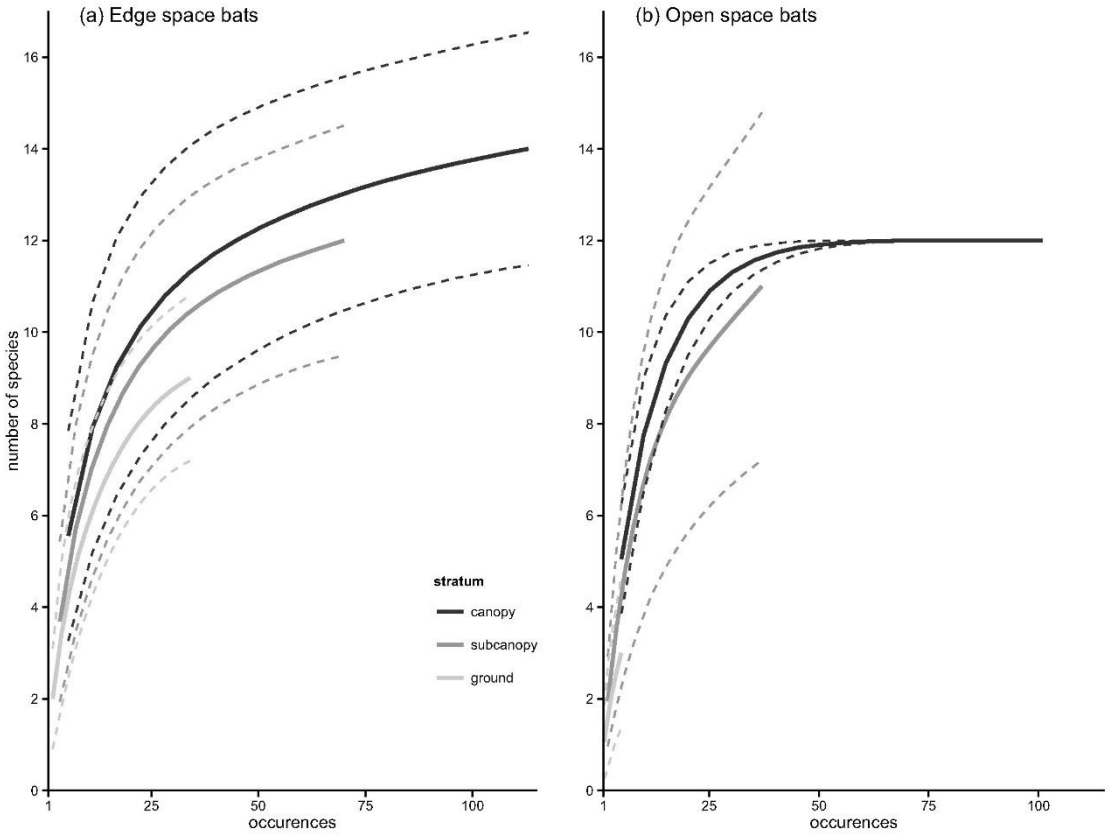

Figure 3.4 Species richness for edge space (a) and open space bat species (b) in forest interior, at ground, subcanopy and canopy levels (solid lines) with 95% CI (dashed lines). Data were rescaled to occurrences.

Influence of riparian vegetation on vertical use of space

The analysis of the activity bats at riparian sites, near the edges of lakes and streams, demonstrated that in these habitats the vertical pattern of use of space was very different from that observed in the forest interior; overall activity was concentrated at water surface level and decreased towards the canopy level (Figure 3.5a). This pattern was marked and statistically significant in the case of edge space species (χ^2 = 10.59, DF = 2, p = 0.005). The data suggest a similar pattern for open space bats, although it was less marked and did not reach statistical significance level (χ^2 = 1. 56, DF = 2, p = 0.456).

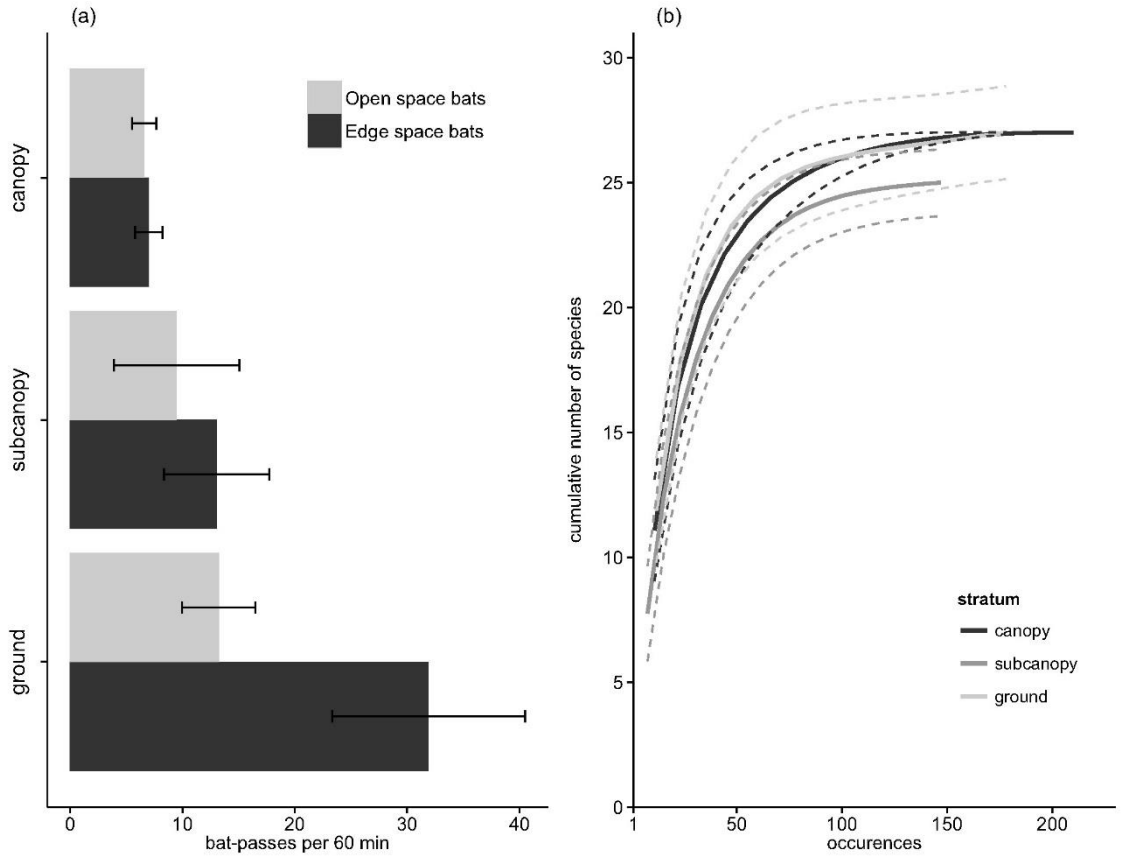

Figure 3.5 Influence of riparian edges on the vertical activity of edge and open space bats (a) and on species diversity (b). Lines in (a) are one standard error of the mean.

In contrast to the situation in the interior of the forest, overall species diversity did not vary across strata (Figure 3.5b).

3.5 Discussion

This is the first study to describe in detail the pattern of vertical use of space by aerial insectivorous bats (*i. e.* families Emballonuridae, Vespertilionidae and Molossidae) in tropical rainforest. There are a number of studies describing the use of vertical space by bats based on captures with mist nets (Kalko and Handley 2001, Ramos Pereira *et al.* 2010), but insectivorous bats tend to be difficult to capture and are thus poorly represented in these studies. Our study uses data collected with ultrasound recording stations, which are far more efficient than nets at detecting insectivorous bats, although species with low intensity or highly directional calls may be underrepresented in acoustic surveys. The intensity of calls of most species present in our study area has not been studied, but the information available (Henze and O'Neill 1991, Surlykke and Kalko 2008) suggests that few species have calls below 110 dB, the reference value indicated by Griffin (1958) for high intensity aerial insectivores.

Marked stratification in the use of vertical space by aerial insectivorous bats

Our results revealed a clear vertical stratification in the use of space by aerial insectivores in Amazonian rainforests. In both edge and open space species the

activity at the subcanopy and canopy levels was several times greater than at ground level (Figure 3.6).

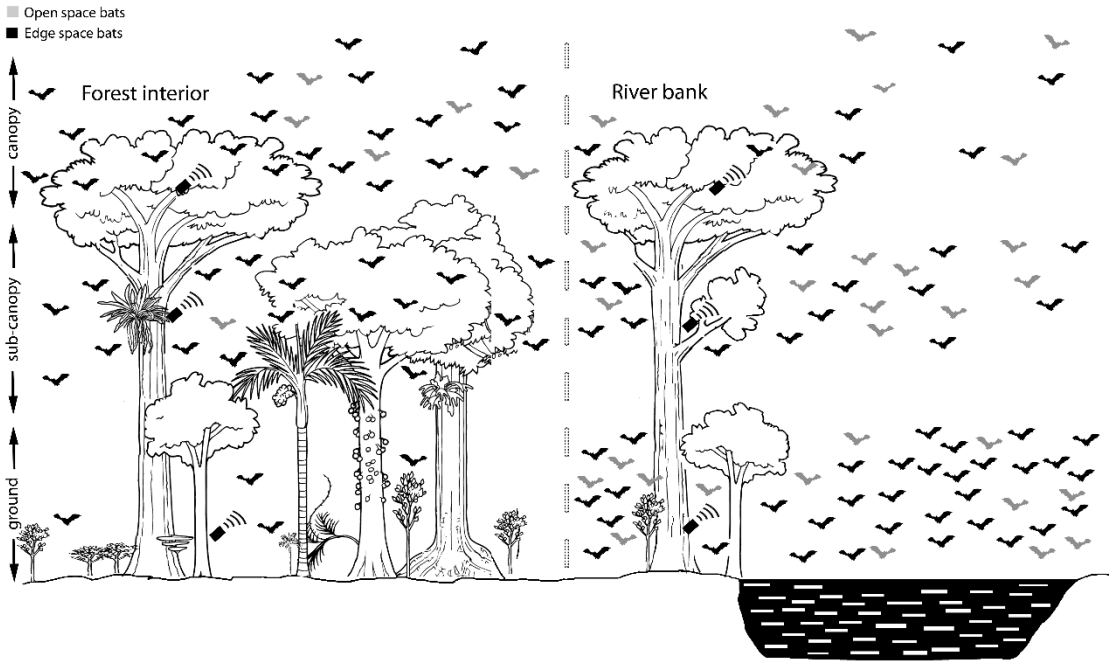

Figure 3.6 Schematic illustration showing the sampling design and main results. Automatic recording stations placed at the canopy, subcanopy, and understorey levels, in both forest interior and along water bodies, sampled insectivorous bat activity. In the forest, both edge space (in black) and open space bats (in grey) were far more active in the upper layers, but this pattern was much more accentuated in the latter species group; open space bats were virtually absent from the ground level. Most bats seem to concentrate their activity in the open spaces between canopy layers, or above the canopies. These patterns were altered at the edges of water bodies, where air space is less cluttered. Here bats tend to be more active close to the ground level.

This concentration of bat activity at the upper strata was particularly marked for open space species; all twelve open space species were recorded more frequently in the stations placed at canopy and subcanopy levels, and were virtually absent from the ground level recordings. This is unsurprising because open space bats, which include some emballonurids and all molossids, are known to mostly fly high over forested habitats when commuting or foraging. They have long narrow wings, which results in a fast flight with low manoeuvrability (Norberg and Rayner 1987), and their echolocation,

based on long pulses, is particularly suitable to use in the wide open space above the canopies (Schnitzler and Kalko 2001). In fact, the occurrence of these bats in the subcanopy may be less frequent than our results suggest, because some strong echolocation calls emitted above the canopy may have been recorded in the subcanopy stations.

However, our results show that the overall increase in aerial insectivore activity in the upper strata is not just due to a greater presence of open space bat species. In fact, an analysis excluding them confirmed that the activity of edge space bat species is also much greater in the canopy and subcanopy than near the understorey. This concentration of the activity of edge space bats (all vespertilionids and some emballonurids) in the upper forest strata was a somewhat surprising result; on average their activity was four to five times greater than near the ground. With the exception of *Myotis riparius*, which was more frequently recorded at the lowest stratum, all edge space species preferred to forage at either the canopy or the subcanopy levels. M. riparius is one of the smallest of the studied bats, and its reduced size and echolocation features, short duration pulses (~2.5 ms) and a high repetition rate (inter-pulse interval ~57 ms), indicate that it can forage very close to understorey vegetation (Norberg and Rayner 1987, Fenton *et al.* 1999, Schnitzler and Kalko 2001). The reduced use of the low forest strata by most aerial insectivores is likely to release resources for *M. riparius*.

In addition to aerial insectivores, the assemblage of insectivorous bats of Neotropical rainforests includes several species of insect-eating Phyllostominae, a sub-family of the phyllostomids. These are more easily captured in mist nets, and several studies have shown that they use the vertical space quite evenly; some species also prefer to forage near the canopy but a large proportion forages mainly in the understorey (Kalko and Handley 2001, Bernard 2001, Ramos Pereira *et al.* 2010, Rex *et al.* 2011). In contrast with aerial insectivores, these Phyllostominae glean most of

their insect prey from vegetation surfaces. Their adaptations to this foraging mode include broadband short echolocation calls (Schnitzler and Kalko 2001) and a slow and manoeuvrable flight (Norberg and Rayner 1987), both needed to efficiently forage in cluttered environments.

Neotropical forests also harbour many species of frugivorous and nectarivorous bats of the family Phyllostomidae, and their use of vertical strata in the forest is well known. Some species seem to forage mostly either in the canopy or in the understorey, but several use the strata more evenly (Kalko and Handley 2001, Bernard 2001, Ramos Pereira *et al.* 2010). Like the insect eating Phyllostominae they have adaptations to fly close to vegetation (Marinello and Bernard 2014).

In conclusion, the preference of aerial insectivorous bats for foraging in the upper strata of the forest is clear and contrasts with the patterns observed with other Neotropical forest bat guilds, which include species better adapted to fly in confined spaces.

Why do bats prefer to forage at the canopy and subcanopy levels?

While it is obvious that open space bats concentrate their activity above the canopy because they can only forage in wide-open spaces, the reasons for the very strong vertical stratification that we observed in edge space bats are less evident.

A greater abundance of insect prey in the canopy may help to explain the preference of aerial insectivorous bats to forage in the upper strata of the forest. Information on vertical stratification of the overall arthropod abundance in tropical rainforests is scarce, but the few existing studies indicate that they are more abundant

in the canopy (*e. g.* Adis 1997; Basset 2001). This is to be expected because food resources for phytophagous insects, such as fruits, flowers and green foliage, are usually more abundant in the canopy (Barrios 2003), which also has a greater diversity of niches than the understorey (Smith 1973, Parker 1995). However, there are also groups of arthropods that do not show differences between strata or are even more abundant and species rich at ground level. Some studies found the latter patterns in Diptera and Lepidoptera, two of the insect groups most consumed by bats (De Djin 2003, Brehm 2007). Therefore, prey abundance alone may not explain the great concentration of aerial insectivorous bats near the canopy.

It has been demonstrated that many predators, including some bats, do not necessarily hunt in the habitats with the greatest abundance of prey, because environmental factors may hinder their capacity to capture prey (Brigham *et al.* 1997, Rainho *et al.* 2010). In fact, the density of obstacles was the main factor explaining variation in the levels of activity of aerial insectivores in Panama (Estrada-Villegas *et al.* 2012). In tropical rainforests the amount of vegetation clutter varies vertically. It is usually particularly dense near the ground due to the abundance of tree saplings and shrubs, but the layer between the undergrowth and the canopy often includes large air spaces. In addition, there are numerous gaps between tree crowns, because trees vary in height (Koike and Nagamitsu 2003, Clark *et al.* 2008). These foliage-free spaces between and below tree crowns are clearly more suitable for foraging by hawking bats than the dense ground layer. They combine high insect abundance and obstacle free spaces needed for bats to capture aerial prey in flight, while providing some protection from aerial predators. This combination may explain the much greater abundance of aerial insectivorous bats foraging at canopy and subcanopy levels that we observed. In fact, Kalko (1995) observed *Saccopteryx bilineata*, *S. leptura* and *Cormura brevirostris* making frequent use of the gaps between strata.

Our interpretation that aerial insectivores forage much more at canopy and subcanopy levels than at ground level mostly because of greater clutter in the latter, is further corroborated by the fact that *M. riparius* is the only species of this guild that forages mostly at understorey level. As referred above this is presumably the aerial insectivore best adapted for foraging in confined spaces. Further studies are needed to evaluate the potential role of additional factors that may explain the concentration of foraging bats in the upper strata of rainforests, such as the height of day roosts.

Stream edges alter vertical use of space

The overall activity of aerial insectivorous bats along the vegetation of stream banks and lakes was twice that recorded in the forest interior, and several factors may contribute to this difference. First, riparian vegetation and water bodies are usually highly productive habitats, with greater abundance of insect-prey than the interior forest (Iwata *et al.* 2003, Fukui *et al.* 2006, Chan *et al.* 2008). Second, even bats that forage away from water bodies have to visit them for drinking (Russo *et al.* 2012). Finally, bats are known to use obstacle free linear spaces to commute while foraging (Palmeirim and Etheridge 1985; Meyer *et al.* 2005), and the spaces over water courses are easily travelled flyways.

Aerial insectivores not only use riparian sites more than the forest interior, but also use its vertical space very differently. The greatest contrast is that observed in edge space species, which reverse the way they use the vertical space and are far more active at low levels than near the canopy. Even open space bats, which in the forest tend to fly above the canopy, change their behaviour and show similar levels of activity across the three height strata. Over streams and lakes clutter does not impose

significant constrains to foraging, so the vertical use of space by foraging bats may be determined mostly by insect abundance, which is usually greater close to the water surface.

Implications for conservation and future studies

The fauna using the upper strata of rainforests has remained poorly studied, mostly because it is usually much more difficult to sample at canopy level than near the ground. However, new sampling techniques are revealing that the main centre of activity of many animal groups in the rainforest is the canopy (Heymann *et al.* 2002), as we now observed for aerial insectivorous bats. In fact, the type of sampling that we carried out only recently became feasible with the development of stations that automatically record bat calls, a technique that in spite of its shortcomings is providing valuable information on bat activity and on the structure of species assemblages (Plank *et al.* 2012, Müller *et al.* 2013).

Our results underline the importance of including canopy sampling in ecological studies of rainforest aerial insectivorous bats. In fact, making observations just at ground level often means that we are studying a species in a peripheral habitat, which may lead to biased results or make them less relevant. In addition, sampling the activity of aerial insectivores using bat detectors at ground level is far less efficient than close to the canopy; at ground level we recorded seven times fewer bat passes and half the number of species than in the canopy (Figure 3.2 and Figure 3.3), even though we used a similar sampling effort in both strata. In rainforest some species, particularly high flying open space specialists, are virtually undetectable at ground level (Figure

3.3) because foliage often attenuates their calls; these species are likely to be missed if activity is not monitored near the canopy.

Our observation that in Amazonia most aerial insectivores concentrate foraging at canopy and subcanopy levels probably also applies to other rainforests. In fact, rainforests with open spaces between strata occur throughout the humid tropics, and all their assemblages of insectivorous bats integrate a large number of species of aerial insectivores (Kingston *et al.* 2003). However, it would be desirable to confirm this with empirical data, because in the Paleotropics there are bat families not present in the Neotropics (Rhinolophidae, Craseonycteridae, Nycteridae, Megadermatidae) (Findley 1995).

The great concentration of bat activity in the upper forest strata underlines the importance of keeping a well preserved canopy layer for the conservation of aerial insectivorous bats, which are very sensitive to forest fragmentation (Vetter *et al.* 2011). In fact, even subtle changes in the canopy like the low levels of disturbance produced by selective logging (1-4 trees per ha) (Peres *et al.* 2006, Peters *et al.* 2006) may break down the vegetation stratification and thus eliminate inter strata air space. It has been demonstrated that the presence and abundance of aerial insectivores respond to these small changes in forest physiognomy due to selective logging (Peters *et al.* 2006). Open space bats are probably not very affected by such disruptions of the canopy structure, because they fly mostly over the forest. However, edge space insectivores in the absence of the clutter free spaces in the upper forest layers may have a limited access to resources, or be forced to forage in open areas where they may be more exposed to predation (Russo *et al.* 2011). This may reduce their importance as predators of forest arthropods and regulators of insect herbivory.

3.6 Acknowledgements

We dedicate this work to the memory of our colleague and forever friend Elisabeth Kalko whose personal and professional qualities guided numerous bats researchers, made critical contributions to the study of Neotropical bats, and gave us invaluable support in the identification of the bat calls. Kirsten Jung kindly provided advice on the identification of bat calls. The Brazilian CNPq and Ministério do Meio Ambiente issued research permits. João Valsecchi, Helder Queiróz, Miriam Marmontel, Pedro Santos, Jerry Tavares dos Santos, several field assistants, and the staff of the Instituto de Desenvolvimento Sustentável Mamirauá provided invaluable help with planning, logistics and fieldwork. This study was supported by Fundação para a Ciência e Tecnologia (POCI-PPCDT/ BIA-BDE/ 60710 / 2004, co-financed by the ERDF) and a Bat Conservation International grant. MJRP and JTM were supported by FCT doctoral and postdoctoral grants (SFRH/BD/19620/2004, SFRH/BD/22829/2005, SFRH/BPD/72845/2010). Two anonymous referees made valuable comments and suggestions that helped to improve an earlier version of the manuscript.

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Supporting Information

Supplementary material (Appendix EXXXXX at < HYPERLINK "http://www.oikosoffice.lu.se/appendix"www.oikosoffice.lu.se/appendix>. Appendix 1

Appendix 1 - Echolocation sequences during search flight of sonotypes used in this study.

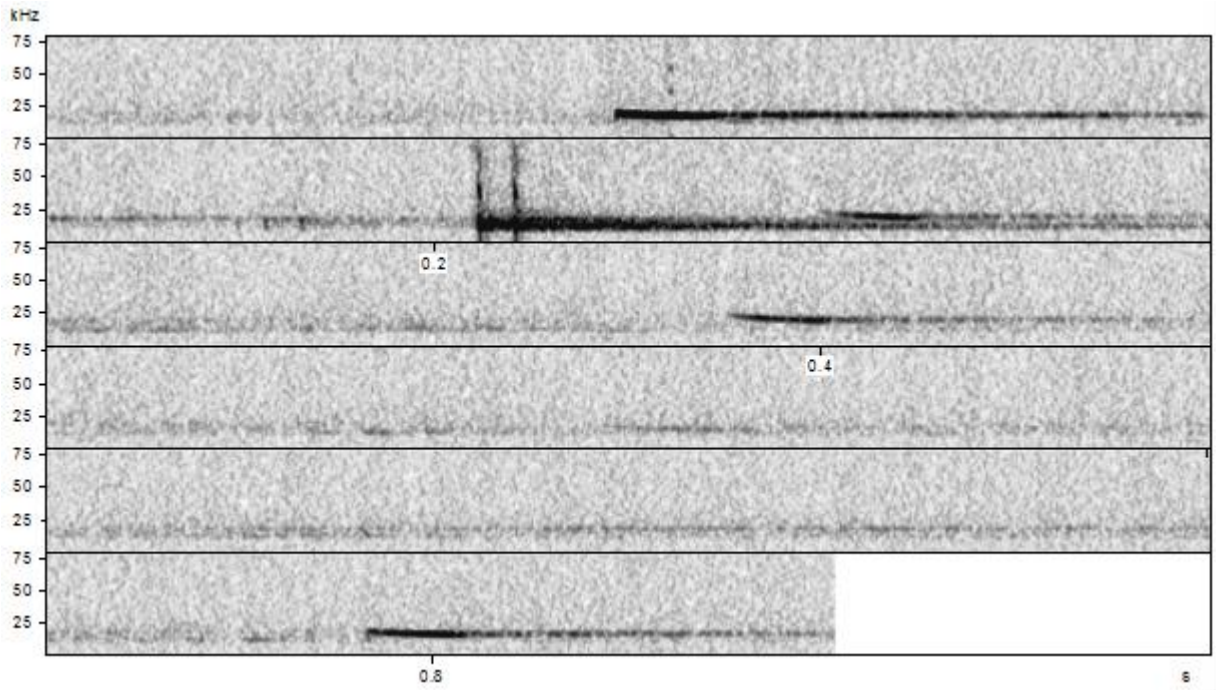

Figure 3.7 Echolocation sequence during search flight of sonotype Large *Eumops sp.* (all Eumops with peak frequency 16-21 kHz).

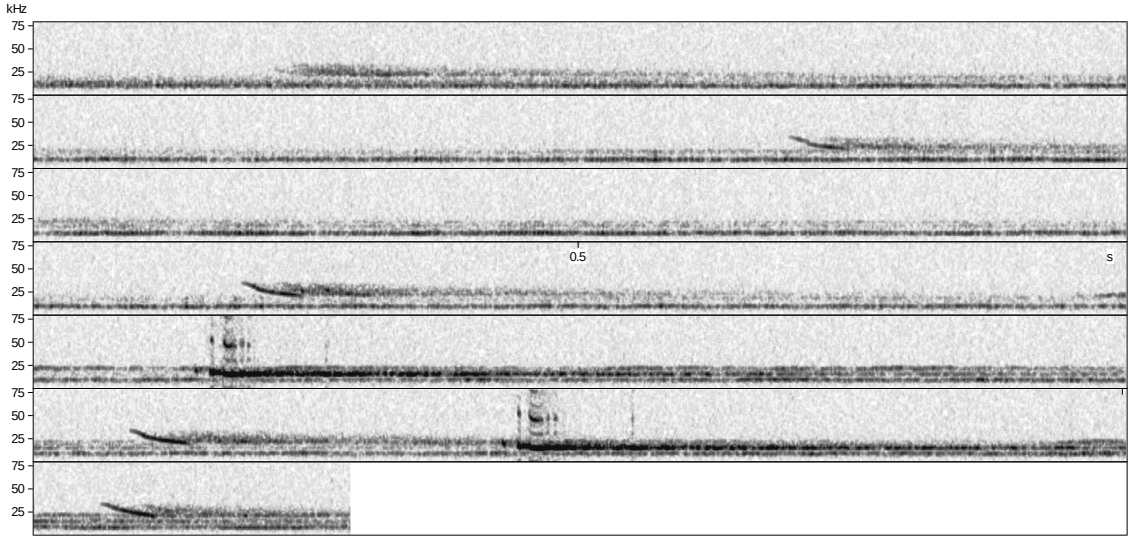

Figure 3.8 Echolocation sequence during search flight of sonotype *Cynomops sp.*

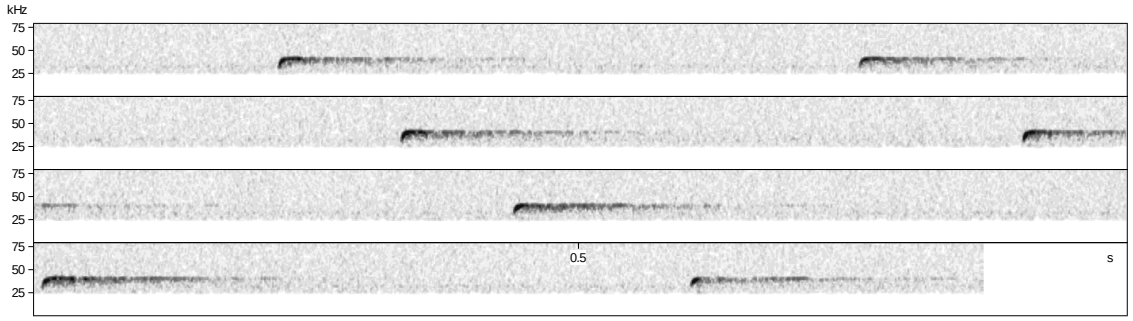

Figure 3.9 Echolocation sequence during search flight of sonotype *Centronycteris sp.*

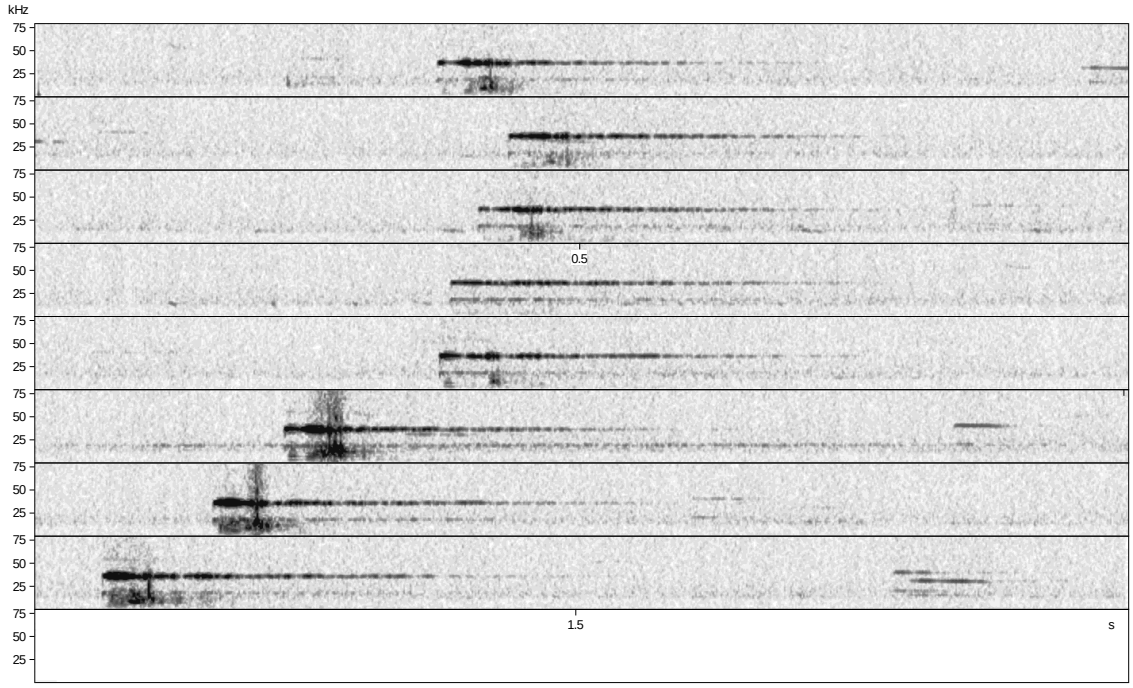

Figure 3.10 Echolocation sequence during search flight of Diclidurus sonotype 1 (with a peak frequency about 36kHz).

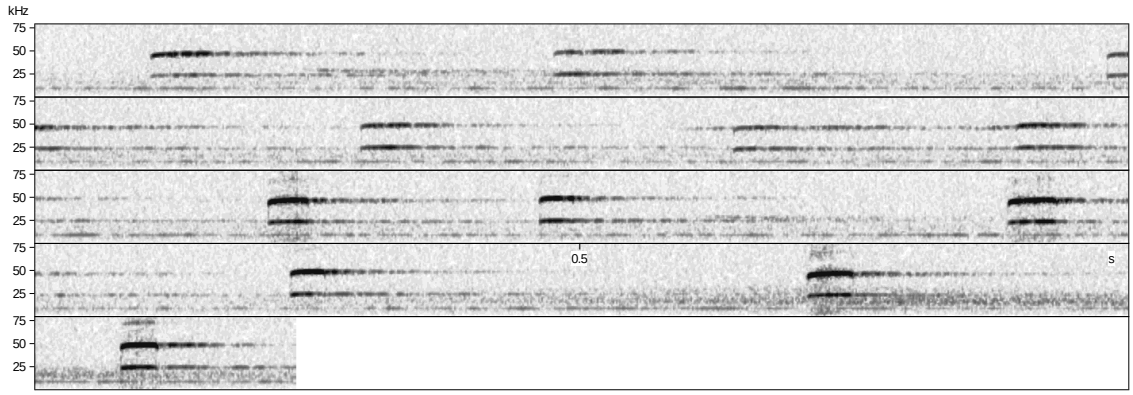

Figure 3.11 Echolocation sequence during search flight of Saccopteryx sonotype 1 (with peak frequency alternating between 45 and 48 kHz).

Chapter 4

Optimizing Sampling Design to Deal with Mist-net Avoidance in Amazonian Birds and Bats

J. Tiago Marques, M.J. Ramos Pereira, Tiago A. Marques, Carlos David Santos, Joana Santana, Pedro Beja and Jorge M. Palmeirim (2013) PLOS ONE 8 (9): e74505

4 Optimizing Sampling Design to Deal with Mist-net Avoidance in Amazonian Birds and Bats

4.1 Abstract

Mist netting is a widely used technique to sample bird and bat assemblages. However, captures often decline with time because animals learn and avoid the locations of nets. This avoidance or net shyness can substantially decrease sampling efficiency.

We quantified the day-to-day decline in captures of Amazonian birds and bats with mist nets set at the same location for four consecutive days. We also evaluated how net avoidance influences the efficiency of surveys under different logistic scenarios using re-sampling techniques.

Net avoidance caused substantial declines in bird and bat captures, although more accentuated in the latter. Most of the decline occurred between the first and second days of netting: 28 % in birds and 47% in bats. Captures of commoner species were more affected. The numbers of species detected also declined.

Moving nets daily to minimize the avoidance effect increased captures by 30% in birds and 70% in bats. However, moving the location of nets may cause a reduction in netting time and captures. When moving the nets caused the loss of one netting day it was no longer advantageous to move the nets frequently. In bird surveys that could even decrease the number of individuals captured and species detected.

Net avoidance can greatly affect sampling efficiency but adjustments in survey design can minimize this. Whenever nets can be moved without losing netting time and the objective is to capture many individuals, they should be moved daily. If the main

objective is to survey species present then nets should still be moved for bats, but not for birds. However, if relocating nets causes a significant loss of netting time, moving them to reduce effects of shyness will not improve sampling efficiency in either group. Overall, our findings can improve the design of mist netting sampling strategies in other tropical areas.

4.2 Introduction

Birds and bats make up a great proportion of the vertebrate diversity in most terrestrial biomes. Both groups are particularly diverse in Neotropical rainforests [1,2], so their study is essential to understand the functioning of these complex ecosystems. Many autecological and community studies in both groups require the capture of individuals, and mist netting has been extensively used for this purpose (*e. g.* [3–6]).

In the case of birds, species surveys are partly dependent on mist netting (*e. g.* [5,7–9]), because in low visibility environments it complements visual and auditory methods. Netting efficiently detects secretive species and is not affected by inadequate knowledge of local bird calls or observer bias [10]. In addition, it has been demonstrated that, when used correctly, mist netting is a safe method to capture birds [11]. Bat studies are even more dependent on the use of mist netting, and almost all sampling of Neotropical forest bat assemblages has used this technique [6,12]. Surveys using recordings of bat echolocation calls are becoming increasingly sophisticated [13,14] and a few have been done in the Neotropics [15–17]. However, the results of these surveys depend greatly on the techniques and technology used [18], and the identification of the species emitting the calls is often difficult because of poor knowledge about the echolocation calls of most species and overlap in call structure [19]. In addition, Neotropical bat assemblages are dominated by Phyllostomids, which have calls that are difficult to detect in the field [20,21].

The advantages of mist netting and the shortcomings of alternative methods warrant that netting will remain an essential technique in ecological studies of Neotropical birds and bats. However, one of its major drawbacks is that both birds and bats appear to learn the location of nets and thus avoid them, a phenomenon usually

referred to as net avoidance or net shyness (*e. g.* [22–24]). It has been demonstrated that when nets are placed in the same location for consecutive days, net avoidance usually results in a substantial decline in captures over time [7,25], leading to a decrease in the efficiency of sampling. The reduction in the numbers of captures can affect not only data collection about individual species or groups of species but also in community surveys, because the drop in captures usually results in the detection of fewer species [25].

Changing the locations of mist nets every day has been recommended as a strategy to avoid the decay in captures in both bats [26] and birds [8]. However, in some situations moving the nets may result in a loss of netting time, because the amount of work setting up mist nets at new sites can be substantial. This is the case if new suitable sites have to be selected, and net lanes have to be cleared for a large number of nets, or when using canopy nets, as their deployment is very time consuming [26]. For this reason, researchers need to weigh the advantages of moving the nets to avoid shyness against the consequences of loosing netting time.

In Neotropical studies the number of consecutive days with nets in the same locations is highly variable, both in birds and bats [6,8]. A few of those studies quantify the day to day decay in the number of captures [25,27], but they do not quantify the consequences of avoidance on the numbers of species detected. In addition, to our knowledge there are no data studies that evaluate the consequences of net avoidance on the efficiency of surveys.

The overall objectives of this paper are to: (i) quantify and analyse the effect of mist net avoidance on captures of Neotropical birds and bats, (ii) determine how net avoidance influences the efficiency of bird and bat surveys under different logistic scenarios, and (iii) formulate advice for designing sampling strategies that minimize the impact of net shyness on bird and bat sampling.

4.3 Materials and methods

Study area

Field work took place in the Amanã Sustainable Development Reserve (2˚37'S, 64˚37'W, Amazonas, Brazil), between April and December 2007. The Reserve includes over 2 million ha of forest including some that are seasonally flooded with nutrient-rich "white" water, known as várzea forests, and nutrient-poor "black" water, known as igapó forests [28,29]. Canopy height varies among the three forest types but is usually between 15 and 35 m, with emergent trees often reaching 50 m [29]. The area receives about 2500 mm of annual precipitation, mostly during the high-water season, from January to June. The low-water season is usually between July and December. Water levels in flooded forests vary up to 10 meters between the two seasons [29].

Bird and Bat Mist-netting

We captured birds and bats at a total of ten sites; four in non-flooded forest, three in igapó and three in várzea (for details, see [3,4]). Each site was sampled in both the high-water and low-water seasons, resulting in a total of 20 sequences of four consecutive mist netting days. We assumed that birds and bats forget the location of the nets between the two seasons, because the time between visits averaged 173 days (range: 128 - 231), much longer than the three week interval recommended by Bierregaard [7]. In the high-water season nets were set just above the water in both várzea and igapó.

In each sampling site and occasion we used 10 mist nets (12 x 3 m; 5 shelves, Denier 110, 16 mm mesh size) for capturing both birds and bats in the forest understory. The same nets were opened for four consecutive days at the same locations between 6:30 to 11:00 and 16:30 to 18:30 (for birds), and between 18:00 to 24:00 (for bats), except when raining. Nets were checked every 30 min for birds and every 20 min for bats. All captured birds were identified, aged, sexed and marked by clipping the tip of the third primary of the right or left wing in the high- and low-water seasons, respectively. Bats were sexed, weighed and identified using the key by Lim & Engstrom [30] and an unpublished key by Erica Sampaio and Elisabeth Kalko. We marked the wing membranes of bats using a pen to recognize recaptures during the same four-day sequence. Our protocol was approved by the Brazilian CNPq and Ministério do Meio Ambiente.

Testing the Effect of Capture Decay

We examined the trend of captures over four consecutive days using Generalized Estimating Equations (GEE; [31]) because of their suitability to analyse temporally correlated data. Rather than choose a specific correlation structure for the few (four) consecutive days sampled at each site, we used robust (and empirical) sandwich estimates of variance based on the correlation observed within sites to determine the standard errors for model parameters and any associated tests of significance, using the R package geepack [32].

Influence of Species Abundance on Net Avoidance

We tested the relationship between species abundance and capture decay for all species with more than seven captures using a two-step approach. First, for each species, we determined the linear trend in captures over the four netting days using the pooled data of the 20 sampling sequences. Capture numbers where standardised (centred and divided by the standard deviation) to compare species with very different numbers of captures. Species with greater decay in captures have steeper trend slopes. We then tested the linear relationship between all these species-specific slope values and the logarithm of the numbers of animals captured. The significance of the relationship was determined by Ordinary Least Squares using PAST software - version 2.17b [33]. We assumed that the number of captures of each species is proportional to

their local abundance in the forest understory, although this relationship is only approximate because it is affected by several confounding factors [34].

Impact of Mist Net Avoidance on Sampling Efficiency

Mist net avoidance may affect sampling efficiency both by decreasing numbers of animals captured and the number of species detected. The decay in captures due to avoidance is likely to increase with the number of consecutive days that nets remain at the same locations. We quantified the effect of this decay for sampling strategies with nets remaining one, two, three and four consecutive days at the same locations, by restricting the capture data to the number of netting days that we would use in each strategy. For the one-day strategy (1-day), *i. e.* in the absence of net avoidance, we used the results of the first day of captures of the 20 sequences. For the three remaining strategies, which are presumably affected by increasing net avoidance effect, we used the first and second days of captures (2-day); the first, second and third days of captures (3-day); and all four days of captures (4-day). Using these values we then simulated a field season with 24 days and compared the results of the four strategies. We also analysed two scenarios: (i) when moving the nets to another sampling site does not imply the loss of netting time, and (ii) when moving the nets requires one field working day, as is often the case in logistically difficult study areas or when using canopy mist-nets.

To evaluate sampling success in terms of species captured we compared the efficiency of the four survey strategies – 1-day, 2-day, 3-day and 4-day – with sample based species rarefaction curves. Calculations were done using the Mao Tao estimator on EstimateS (v. 8.2.0, [35]). The rarefaction curves were extrapolated to a total of 24

survey days using an estimator based on the Bernoulli product model, proposed by Colwell *et al.* [36]. The number of species present in the assemblage but not observed in any of the sampling units of the reference sample was obtained with the Chao2 estimator [37,38]. All calculations were done separately for the three types of forest (non-flooded, várzea and igapó) and the results averaged.

4.4 Results

Quantification of the Decay in Captures

Decay in captures with nets at the same locations was observed in both birds and bats, although it was greater in bats (Figure 4.1). Captures over the 4-day period declined by 68% in bats and 45% in birds. Both declines were statistically significant (bats $p<0.001$; birds $p=0.013$) and occurred mostly between the first and the second days of mist netting.

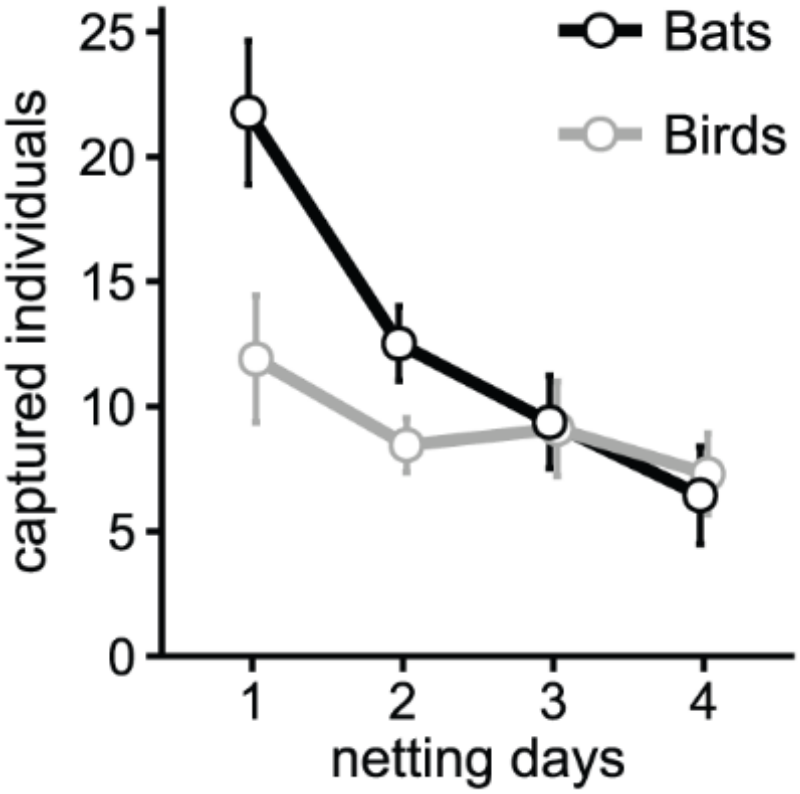

Figure 4.1 Decay in mist-net capture numbers of birds and bats. Mean daily capture numbers of bats and birds over four consecutive days with mist nets at the same location. Lines connect the average values over consecutive days. Data were pooled across seasons and forest types. Vertical lines represent 95% CI (n = 20).

Relationship between Species Abundance and the Decay in Captures

The decay in captures was most evident in the common species of birds (Figure 4.2A) and bats (Figure 4.2B). This relationship was statistically significant for both groups (bats $r = -0.49$, $p = 0.02$; birds $r = -0.46$, $p = 0.005$).

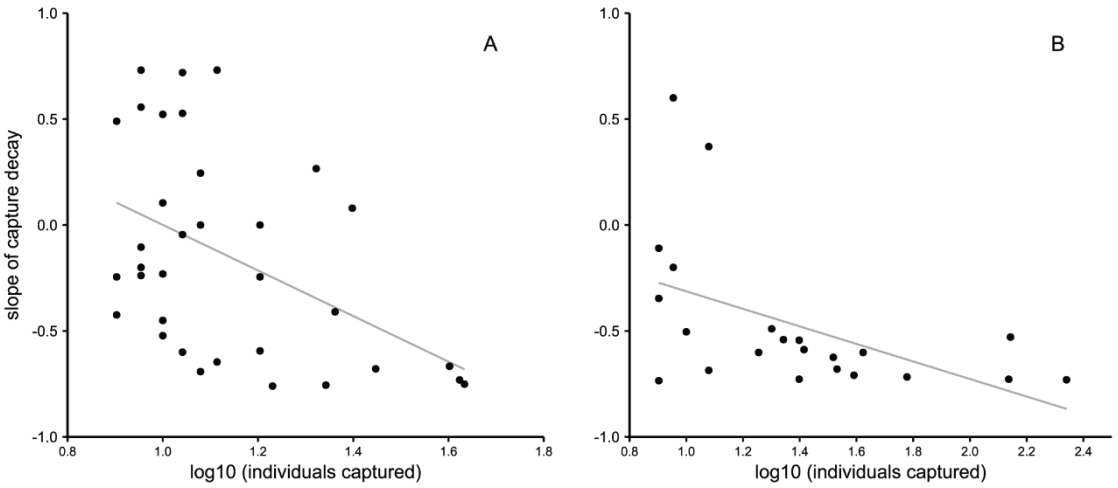

Figure 4.2 Relationship between species abundance and the decay in captures of birds (A) and bats (B). Data are the slope of the decay of captured individuals for each bird (A) and bat species (B) over 4 nights. The most abundant species tended to have a more accentuated decay.

Influence of Net Avoidance on Bird and Bat Sampling

The comparison of the four sampling strategies (1-day, 2-day, 3-day and 4-day) shows that net avoidance had a strong impact on the efficiency of bird and bat surveys in terms of total number of captured individuals (Figure 4.3). For the same overall survey duration we captured fewer animals if nets were deployed for more days at the same location. The drop in efficiency was greater for bats than for birds (Figure 4.3 a, b). For example, by changing the location of the nets daily at the end of 24 mist netting days we estimate we would have captured about 286 birds, whereas having the nets in the same location during four days would result in 221 captures, *i. e.* a 23% loss in efficiency. The same comparison in bats would result in an estimate of 522 versus 301 captures, a 42% drop in efficiency. However, this drop in efficiency only occurs if the locations of the nets can be changed without missing any mist netting days.

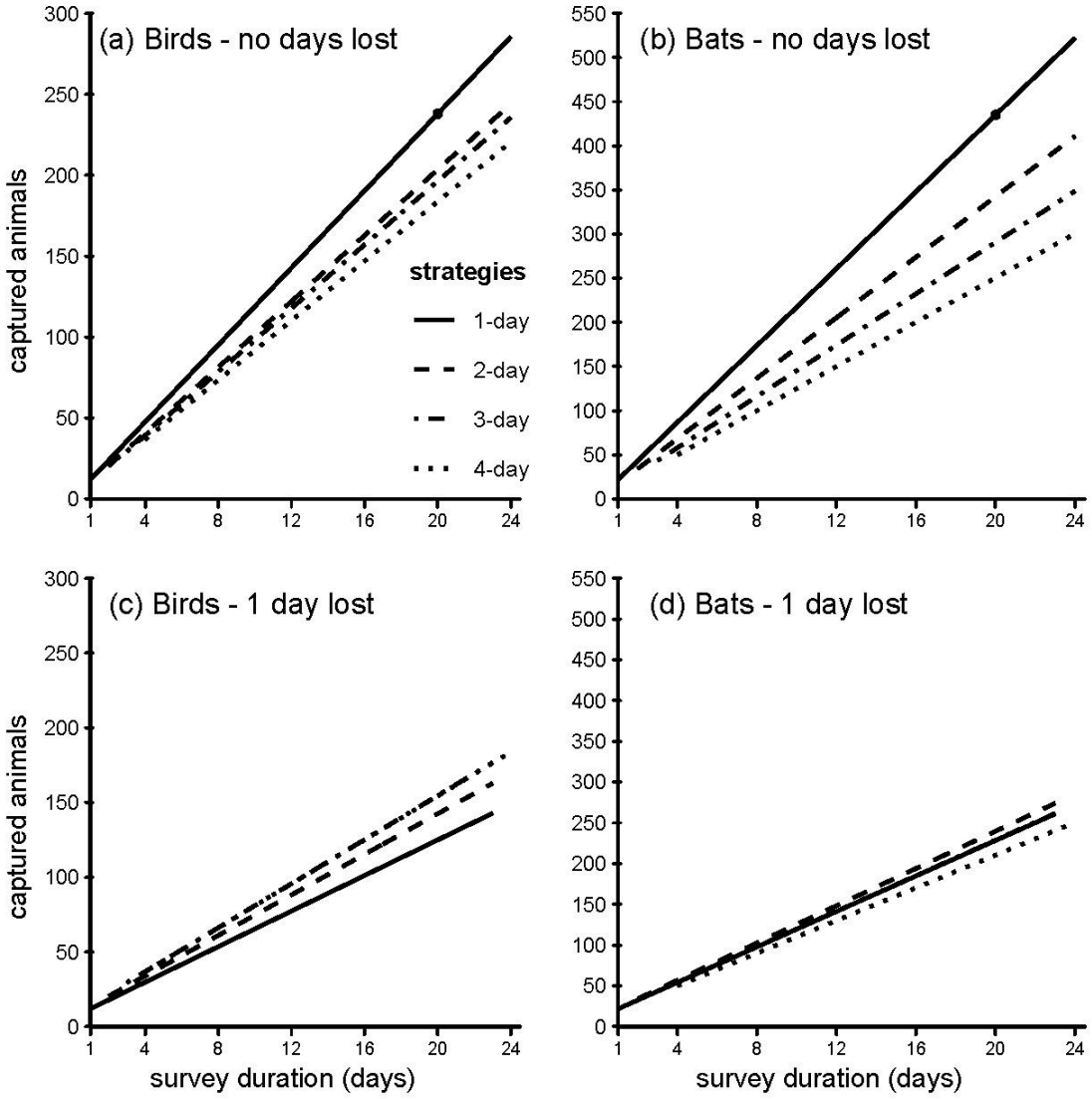

Figure 4.3 Capture numbers of birds (a,c) and bats (b,d) using different sampling strategies. Mist-net captures of birds and bats in simulated surveys lasting up to 24 days when nets were moved daily or remained at the same location 2, 3 or 4 days. When nets are set up in the same locations for 1 to 4 consecutive days net avoidance causes an increasing decline in the total number of animals captured in the survey of birds (a) and especially in bats (b). But whenever moving the nets involves losing one netting day per site, net avoidance does not affect the total numbers of animals captured in the survey of birds (c) and bats (d). The line representing the 1-day strategy was extrapolated to the right of the dot.

If moving the nets requires even just one field working day, as is often the case in logistically difficult study areas or when using canopy nets, then moving the nets daily may no longer be an advantage. For birds, the decrease in efficiency due to the loss of netting days is greater than the loss due to net avoidance (Figure 4.3c). We estimate that keeping the nets at each location 3 days one would capture 184 birds in 24 field days, but only 149 if we move the nets daily with the loss of one field day in between. In bats the loss of capture days moving the nets cancels out the advantage of minimizing net avoidance and the results of all four sampling strategies become similar (Figure 4.3d).

In the case of number of species detected, net avoidance had a substantial impact on the numbers for both birds and bats. Species rarefaction curves show that longer stays at each location result in less species recorded during a 24 day mist netting period (Figure 4.4a, b).

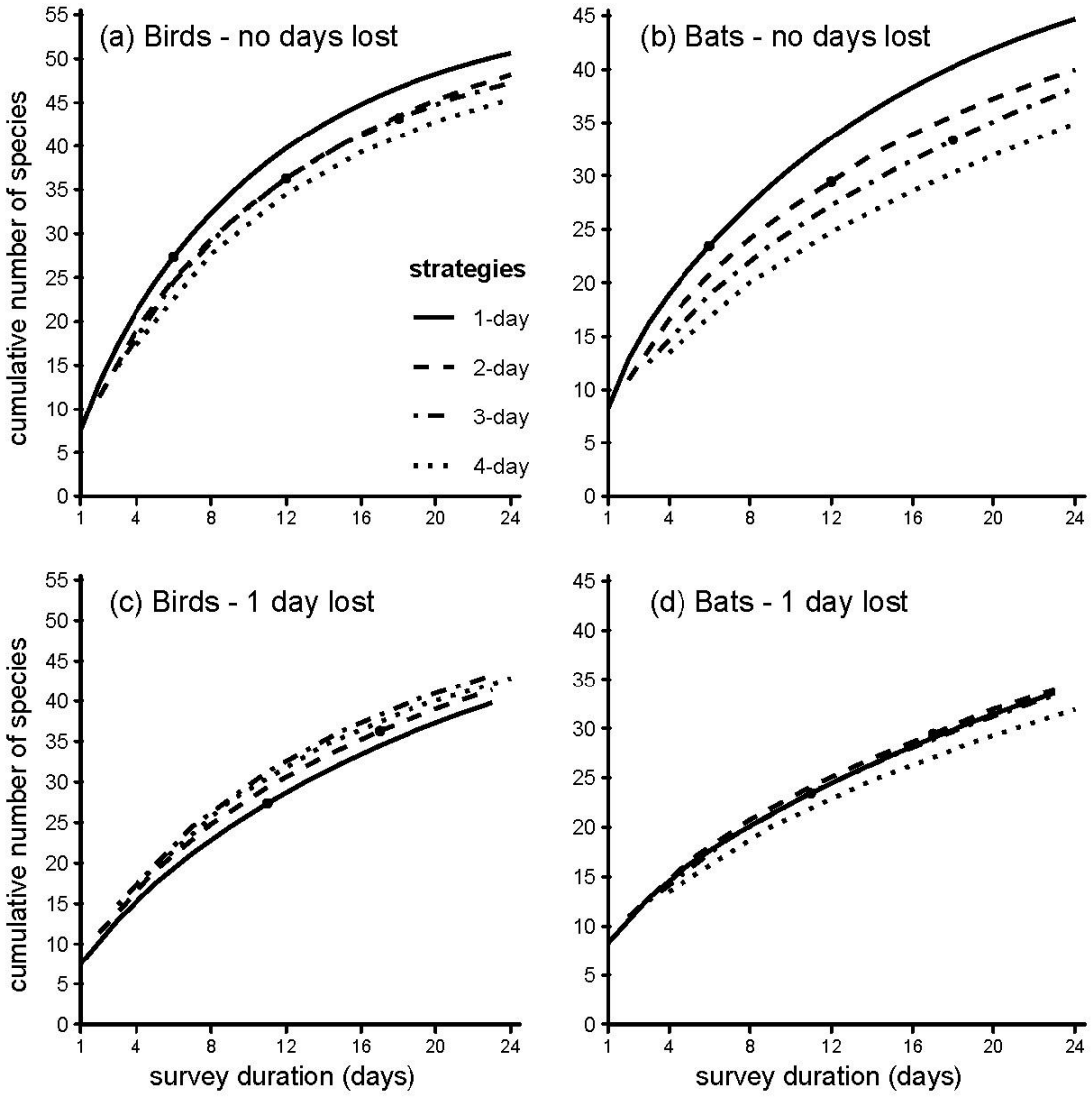

Figure 4.4 Species rarefaction curves for birds (a,c) and bats (b,d) obtained using four sampling strategies. Number of species detected in simulated surveys lasting up to 24 days when nets were moved daily or remained at the same location 2, 3 or 4 days. When nets are set up in the same locations on consecutive days, net avoidance causes an increasing decline in the number of species detected in the bird survey (a) and especially in bats (b). However, when moving the nets involves losing one field working day per site net avoidance does not affect the total numbers of species detected in the survey of birds (c) and bats (d). Above the black dots curves are extrapolated.

We estimate that without loss of days between netting locations only about 45 bird species are detected when the nets remain for 4-days at the same locations whilst moving the nets every day would result in the detection of 51 species, a difference of 12%. Likewise in bats the number of species detected would go up from 35 to 45, *i. e.* a difference of 22%. Again, the advantages of changing net locations daily disappear when moving them requires one field working day per site, because this reduces the time that is possible to dedicate to netting during the 24 day sampling period. In both birds and bats the numbers of species detected are more similar in all sampling strategies (Figure 4.4c, d).

4.5 Discussion

Quantification of the Decay in Captures

The numbers of bats captured with the nets set up at the same locations over consecutive days dropped quickly from day to day. Captures in the second day were almost half of those in the first day, and by the fourth day they were reduced to less than one third. This pattern of decay is in line with that reported in other Neotropical and Temperate studies. Estrada *et al.* [39] and Simmons & Voss [25] reported an average decay of 50-70% between the first and the second day of netting in Mexico and French Guyana, respectively, while Esbérard [40] in South-Eastern Brazil observed a reduction of 65% between the first and third days. A similarly marked decay is evident in the few studies reporting quantitative observations in other biogeographic

regions; for example, in Missouri (U.S.A.), Robbins *et al.* [41] observed a 45% decay between the first and second netting day. It can thus be concluded that net avoidance roughly halves the captures between the first and second day of captures, and reduces them further if nets remain for longer periods at the same location.

Mist net avoidance in Neotropical birds seems to be less accentuated than in bats. We observed an average drop of about 30% between the first and the second day using the same mist net setup. Data suggested a further decline with time, but less pronounced. Few studies report quantitative information on net avoidance in the Neotropics. However, Faaborg *et al.* [27] in Puerto Rico observed a 36% decline in captures between the first and second day, and of 14% from the second to the third day.

Why Is Net Avoidance So Marked in Bats?

Although the captures of both birds and bats declined over consecutive netting days, this decline was much steeper in the latter. In addition, far more birds than bats were recaptured in the same four-day netting sequence (13% vs 0.4%), adding to the evidence that bats are better than birds at learning to avoid previously encountered nets. Which factors may explain such strong net avoidance?

Bats are known to have an exceptionally good spatial memory [42–44], so once they have located a net they can probably avoid it easily. Larsen *et al.* [45] reported lower bat activity next to mist nets on the second and third nights of sampling. The detection of nets by microchiroptera is facilitated by the use of echolocation, which they use constantly or at least while flying in unfamiliar areas [46], but other factors may also help them in this process. It has been demonstrated that bats have a good

capacity for social learning, taking clues from the observation of activities of other animals [47]. Because large numbers of bats tend to use the same commuting flyways [48], it is likely that individuals become aware of the presence of a mist-net by the observation of evasive flights of other bats. They may also locate nets when captured individuals are emitting distress calls, which are often loud and conspicuous [25,49,50].

The spatial ecology of most Amazonian bats may also help explain why they quickly learn the locations of mist nets. The great majority of the bats caught in mist nets in Neotropical rainforests are frugivorous and nectarivorous, and are known to use a trapline foraging strategy, *i. e.* they search for food along regularly used routes inspecting the same potential food sources in a sequential order [49,51]. This repeated use of the same flying routes presumably helps them learn the location of mist nets.

Relationship between Species Abundance and the Decay in Captures

There is little knowledge about the factors that make some species more prone than others to net avoidance. Our results show that in both birds and bats the most abundant species tend to show a steeper decline in the number of captures over time. Faaborg *et al.* [27] reported a similar pattern for birds in Puerto Rico. This is probably related to differences in the way various species use space and how it influences the probability of an individual being captured. Assuming that individuals have some capacity to learn the location of the nets [52], net avoidance should be more accentuated in species whose individuals have a greater chance of encountering a net. The risk of an individual bird being captured is thus potentially greater in species with small home ranges, in which the individual may quickly encounter a net placed within its limits [34]. Captures of these species are thus likely to drop rapidly over consecutive

days of netting. Rarer species tend to have larger home ranges [53, 54], and are thus less likely to encounter a mist net set up in their range within the first day (or days). As a consequence the decay in captures in these species is potentially slower. However, although the correlation between capture decay and the number of captured individuals is significant the relationship is quite noisy. This may be explained by species-specific behavioural aspects that are known to influence net avoidance in birds, such as the species ability to notice, learn and remember the positions of the nets [22], and are also likely to affect bats. In the case of ecosystems with a strong vertical stratification, such as Neotropical rainforests, the way birds and bat species use vertical space [55,56] is also likely to influence their probability of being trapped in mist nets [34].

How to Deal With Mist Net Avoidance?

Field work is often costly and very time consuming, so sampling optimization is important in studies requiring the capture of large number of vertebrates. Although other studies demonstrated that the number of individuals captured can be affected by shyness, this is the first study that quantifies the effect of shyness on the number of species captured. The latter is particularly relevant in studies of the structure of species assemblages. Our results also revealed that shyness does not affect captures of all species equally, and is more severe in the commonest species. The systematic analyses of the consequences of shyness allow us to formulate advice for designing sampling strategies that minimize its impact on bird and bat sampling.

Although shyness affects captures of both birds and bats, the best strategy to minimize its consequences may not always be the same for the two groups. In addition, this strategy depends on the difficulty to reposition the nets within the study area and

on the sampling objectives: *e. g.*, to capture many individuals of common species or to characterize the area's species assemblage.

When the number of mist nets in use is not large and the habitat is relatively open, the location of the nets can be changed quickly enough to avoid any loss of netting time. This is often also possible when nets are set up in pre-existing trails, thus avoiding the need to clear vegetation. In these situations researchers wanting to maximize the number of animals captured should move the nets daily. This strategy would yield gains of about 30% in birds and 70% in bats, compared to keeping nets four days at the same location. Our results indicate that moving the nets daily is particularly important when the aim is to capture individuals of common species, because their captures tend to be more affected by net-shyness.

If the objective of sampling is not to capture many individuals, but to assess the species present in an area, then different approaches should probably be used for sampling birds and bats. For bats it is still best to move the nets daily, because far more species will be detected (Fig. 4B). For birds the gain of moving the nets daily is modest (Fig. 4A). So, the practice of keeping them several days at the same locations, common in Neotropical bird studies [8], is not optimal but does not substantially decrease the survey efficiency. This is explained by the fact that most of the drop in captures is concentrated among the commonest species.

The need to open trails in the forest for the setting up of the mist nets or the installation of canopy mist nets often requires a substantial amount of field work. In these cases moving the nets may result in a loss of netting time and consequently in a reduction of captures. Our results show that if moving the nets causes the loss of one netting day per site then the resulting drop in bird captures is greater than that due to net shyness. Thus, sampling is more efficient if nets are kept at the same sites at least up to three days (Fig. 4C). In the case of bats the loss of captures due to the reduction

in netting time is roughly equivalent to that due to net shyness, so frequent repositioning of nets does not reduce the sampling efficiency, although it also does not improve it.

This study is based on Neotropical data but its conclusions should help the design of sampling strategies elsewhere. This is because the overall drop in captures reported in temperate zones and in other tropical regions is not substantially different from what we observed [22,41,57]. However, researchers should take into consideration that net avoidance varies substantially among species and with environmental factors, and may even be negligible, as in the case of species on migration [22].

Net-shyness is not the only factor to be taken into consideration when deciding how frequently mist nets should be moved. For example, it may be important to maximize the number of independent replicates [58], and using nets at the same location for several days does not allow to treat each day as an independent replicate. Researchers may also want to ponder the negative impact of the clearing of vegetation to set up nets at a greater number of locations, which in some sites may not be negligible. However, it is evident that net shyness affects greatly the efficiency of sampling birds and bats in Neotropical forests, and our results should help researchers to design efficient sampling strategies, thus optimizing the use of limited research resources.

4.6 Acknowledgements

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Chapter 5

General Discussion

5 General discussion

Environmental heterogeneity is in general associated to an increase in species richness. The search for the mechanisms that drive this association started a few decades ago, investigating at several spatial scales, from continental down to plot scales (Stein *et al.* 2014). A myriad of studies assessed and described the general relation between environmental heterogeneity and species richness, but few of those are in tropical and pristine landscapes, particularly at smaller scales (Stein *et al.* 2014). In this thesis I contribute to fill this knowledge gap by researching this topic in the Amazonian forests, using bats as models.

It is generally accepted that three main factors drive the relationship between environmental heterogeneity and species richness, which have been asymmetrically addressed. The contribution of environmental heterogeneity to the increase in niche space availability has been thoroughly studied, while the contributions of environmental heterogeneity for the evolutionary processes of speciation and species persistence have received much less attention (*e. g.* Stein *et al.* 2014).

In fact, environments differ in their ability to support populations (Rickleffs 2004). In the Amazon basin, where there are regions constituted by a mosaic of different types of flooded and terra firme forests, animals can forage in the forest type that provides their preferred resources or with a higher abundance of resources to satisfy their needs. There is mounting evidence that the mosaic of different types of forest in the Amazon, terra firme and flooded forest, supports the co-existence of a high biodiversity in the region for terrestrial and arboreal mammals (Haugassen and Peres 2005a, Haugassen and Peres 2005b). This subject has also been studied for bats and birds, two of the most diverse vertebrates groups in these tropical forests. Species of both groups of flying vertebrates also show differences in the type of forest that they

prefer to live and to forage (Haugaasen and Peres 2007, Ramos Pereira *et al.* 2009, Beja *et al.* 2010). Both Amazonian birds and bats respond to heterogeneity of habitats by selecting different types of forests and adjusting their abundances to the availability of their preferred resources (Ramos Pereira *et al.* 2009, Beja *et al.* 2010, Brobowiec *et al.* 2014). So, there is ample support for an important role of the horizontal component of environmental heterogeneity to the increase of the niche space available. Species coexistence is thus supported by the higher availability of resources provided by the mosaics of forest types in the almost pristine Amazon.

This is further supported by the information published in the paper on Chapter 2. According to our research, the three types of forests, terra firme, várzea and igapó, have different availability of bats' preferred fruits, both for the canopy and ground feeding guilds. As such, both types of forests, flooded and unflooded, contribute to an increase in niche space providing different foraging opportunities for frugivourous bats. These species constitute a significant part of the Amazonian bat community and mammal assemblage (Simmons and Voss 1998). Besides providing further evidence of the role that horizontal heterogeneity plays on the support for local species richness, this information also adds to the mounting evidence of the importance of plant diversity contributing to the species richness of a region (Stein *et al.* 2014).

The vertical dimension is the other main spatial component of environmental heterogeneity; it is often termed habitat complexity (August 1983). Rainforests are one of the earth's biomes where we can observe a more important contribution of this component to the species richness (Fahr and Kalko 2011). The high contribution of the vertical dimension is probably linked to the presence of several strata and results in a marked change in the environmental conditions from the ground to the canopy of these forests.

Flying vertebrates are excellent biological groups for the study of vertical patterns of use in forests because they can easily explore the different vertical strata of these environments. In the Amazon, several examples of the importance of the vertical component in the structuring of the assemblages have been published for birds (Walther 2002) and frugivourous bats (Bernard 2001, Kalko and Handley 2001). In the Amanã forests, a mosaic of terra firme (unflooded), igapó and várzea (seasonally flooded) forests, we tested the vertical stratification of 25 bat species, mainly Phyllostomids (Ramos Pereira *et al.* 2010). We concluded that the majority of species preferred the ground stratum, a few preferred the canopy stratum, and some actually used both strata.

Aerial insectivorous bats are also a large part of the Amazonian bat assemblage, and the topic of Chapter 3 is the assessment and test on the pattern of vertical use in the aerial insectivore bat assemblage. In this assemblage there was a marked preference for bats to be active and to forage in the higher strata; with only one exception, all of the 21 studied bat species had higher activity in the canopy and subcanopy compared to the ground stratum (approximately 2m high). The exception was *Myotis riparius*, the smallest bat species analysed. Bat activity in the canopy was more that six fold that at ground level, and species richness was almost twice that observed at the lower level. The marked vertical pattern found for most of the aerial insectivorous foraging guild implies that this ecological response to the changing conditions - from the ground to the forest canopy - may structure the local assemblages. Moreover, these changes also contribute to the increase in the availability of niches. It is very likely that this pattern is present in other Neotropical forests and rainforest sites across the world. Our results concur with the information that the vertical component of environmental heterogeneity is very important in the structuring of the Amazonian bat assemblages (Kalko and Handley 2001, Ramos

Pereira et al. 2010); it is likely that this component makes a major contribution for the coexistence of a great variety of bat species, both frugivorous and aerial insectivorous.

In both the papers from Chapters 2 and 3, the river bank vegetation plays a striking different role from that of the forest interior. By providing an increase in the available resources for bats, *i. e.* fruits, or by releasing aerial insectivorous bats from the flying constraints imposed by dense forest clutter, these habitat discontinuities may play an important role in species coexistence. Within forests, the clutter-free spaces between forest layers also constitute habitat discontinuities providing open spaces where aerial insectivorous bats can forage more efficiently. Therefore, habitat discontinuities are most likely another important spatial component of the link between species richness and environmental heterogeneity.

The habitat discontinuities can be an extension of the concept of keystone structures (see Tews *et al.* 2004), which can play an important role in providing ecological structures for the survival of individuals and species. These structures contain a keystone element that increases species diversity by its presence and they are embedded in ecosystems that have a comparatively homogeneous vegetation structure (Tews *et al.* 2004). Thus, the species diversity of many similar ecosystems might be influenced by the presence or amount of keystone structures. The discontinuities alter both the microclimate and the availability of resources (Belsky and Canham 1994) and they can be detected by abrupt discontinuities in the species–accumulation curves when sampling transects enter the structure (Tews *et al.* 2004). Other examples of common habitat discontinuities or key structures in Neotropical forests are forest gaps (*e. g.* Belsky and Canham 1994), which are important for plant and animal species diversity (Denslow 1987, Hill *et al.* 2001, Schnitzer and Carson 2001).

In the view of the results in this thesis, and of the information in the literature, I suggest that the presence or amount of habitat discontinuities should be added to the horizontal and vertical components of environmental heterogeneity as determinants of species richness. This additional spatial component is likely driving the ecological processes at a lower spatial scale but it may play a role of similar importance to those of the horizontal and vertical spatial components. Thus, I think that the conceptualization of the contribution of the environmental spatial components to bat species richness can be improved by incorporating a component representing the contribution of habitat discontinuities; one axis that includes the amount of discontinuities present (Figure 5.1).

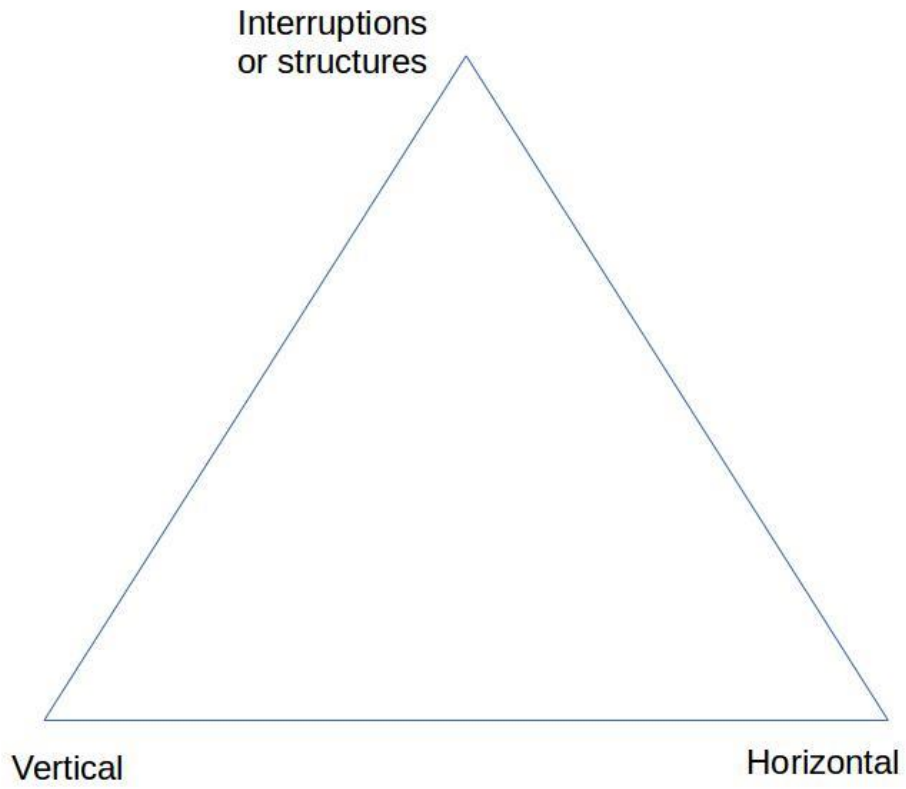

Figure 5.1 Theoretical model of the spatial components structuring species assemblages of bats in the Amazonian rainforests

It is worth noting that, although the concept of key structures or habitat discontinuities was based on the presence of structures, I think that the link between species richness and the amount of habitat discontinuities is probably continuous, with species-specific responses to the gradient of discontinuities.

As stated above examples of these habitat discontinuities can be the edges of forest adjacent to small Amazonian rivers. In some sectors of these margins, the forest is composed of matrix forest that, due to rapid erosion of the bank, becomes virtually adjacent to the river. Also, the spaces between vertical layers of tree foliage in forests constitute discontinuities, that agree to the definition of key structures, *i. e.* they mark the increase of species richness when crossed by a transect. Of course that in this case it would be a vertical transect. Other discontinuities often mentioned in the literature are forest tree gaps (Denslow 1987, Schnitzer and Carson 2001) or their ecological inverse, isolated trees in African grasslands (Belsky and Canham 1994). We believe that both river bank vegetation and open air spaces between vegetation layers are prevailing habitat discontinuities in Amazonian forests and little attention has been devoted to the study of their ecological implications. The spaces between forest strata provide opportunities for foraging bats because they release aerial insectivores from the constraints imposed by vegetation clutter. In rainforests, the spaces between forest layers are also more widespread than forest gaps, which have been subject to numerous studies (*e. g.* Denslow 1987, Hill *et al.* 2001, Schnitzer and Carson 2001).

In conclusion, bats respond positively not only to the horizontal and vertical components of environmental heterogeneity but also to the presence and amount of these habitat discontinuities or disruptions.

The assessment and review of survey methods are important tasks for the scientists relying on them. To know the advantages and disadvantages of different survey strategies, and how to deal with potential biases, are important issues. These

critical assessments of common survey methods often provide new insights (Meyer *et al.* 2011). In chapter 4 of the thesis, we researched the impact on sampling results of a behavioural issue, mist net avoidance by bats and birds. The results show that mist net avoidance affects greatly the sampling efficiency of both birds and bats in Amazonian forests. Strategies to overcome this problem depend on the sampling objectives: to capture many individuals, mist nets should be moved location very frequently, most likely daily; to survey the species present in an area, in the case of bats it is still best to move nets daily but for birds keeping the nets in the same site for several days does not substantially decrease survey efficiency.

5.1 Conservation implications

The new information presented in this thesis underlines the importance of preserving mosaics of habitats even in the most pristine biomes of the world. We found that the food availability for frugivorous bats varies among the three types of forests for both the canopy and the understory guilds. Thus, the landscape composed by these forest types is probably one of the key factors supporting the diverse bat assemblage in the Amazon. Additionally, this mosaic of habitats supports other species rich assemblages such as, those of primates and birds (Haugassen and Peres 2007). Therefore, preserving this mosaic of unflooded and flooded forests is essential to the conservation of biodiversity in the Amazonian forests.

The results in this thesis confirm that, even in a context where multiple types of forest are mosaiqued, features that disrupt the homogeneity of forest, such as river banks and the free spaces between the vegetation layers can increase its capacity to support coexisting species of bats. This agrees with Tews *et al.* (2004) who state that the preservation of the key structures leads to an increase of fitness for the several species that respond positively to them. In terms of biodiversity management this means that conservation of a keystone structure will maintain a higher level of biodiversity, whereas its removal will most likely lead to a decrease in species diversity.

Our results suggest that river bank vegetation is very important for bats foraging in lowland Amazonian forests because these disruptions have more fruits available for bats to eat, both for the canopy and the understory guilds. Moreover, even the slight differences in the forest canopy produced by selective logging can break the vegetation stratification and eliminate the airspaces between layers. These changes can hinder the access of aerial insectivorous bats to resources in the upper strata of the forests

and induce impacts on the presence and abundance of these bats (Peters *et al.* 2006). By foraging in the wider and more exposed areas of the forests these bats may be more vulnerable to predation. Thus, preserving the river bank vegetation and keeping a well preserved forest canopy, including the free spaces between forest layers will support the conservation of the local and regional bat assemblages.

Furthermore, the presence or quality of this habitat disruption or key structure may determine species diversity of one species group. Interestingly, some studies showed that species diversity of several different groups may benefit from the same structural element of the vegetation (Tews *et al.* 2004). In fact, one ecological structure or habitat discontinuity detectable on a specific spatial scale may provide ecological conditions that are relevant for a large proportion of the species groups (Tews *et al.* 2004). Forest river banks provide different resources, and in some cases with greater abundances, than those of the forest matrix, particularly of some key resources such as wild figs. Across tropical forests wild figs are often a key resource for several vertebrate groups (Shanahan *et al.* 2001), including birds (Shanahan and Compton 2001), bats (Kalko *et al.* 1996), and primates (Peres 1994). Thus, in addition to bats, a variety of taxonomic groups can gain from the conservation of this environmental heterogeneity resulting from the presence of different habitats and of habitat discontinuities.

In conclusion, landscape homogenization through the loss of habitats and habitat discontinuities in the Amazonian forests may have direct implications to animal populations that may conduct to species loss.

5.2 Final remarks and further research

The influence of horizontal, vertical and discontinuities components of environmental heterogeneity provides an increase in the resources and gradients available for assemblages of frugivorous and insectivorous bats. These new informations, discussions and conclusions integrated in this thesis raise new research questions and allow knowledge refinements that could further expand our understanding of bat ecology in Amazonian forests.

New techniques emerged recently for the sampling of bat echolocation calls that can help addressing these research questions. In fact, new hardware (automatic bat recording stations) and software (automatic identification algorithms) will further develop and facilitate the study of bat echolocation in tropical forests. However, to be useful, these technical advances need to be matched by an extensive effort to collect and organize libraries of identified bat calls that can be used as reference.

The use of genetic identification techniques can also help in the gathering of new information on the diet of Amazonian bat species. A much broader and precise understanding of bat diets will emerge with the application of these new tools (Clare 2014, Handley 2015). With traditional techniques the consumption of large fruits by bats cannot be detected because their seeds are usually too large to be ingested, and thus do not leave morphologically identifiable remains in the bat faeces. The identification and quantification of the role that bats play in the seed dispersion of large fruits is essential to study how this biological group contributes to the maintenance and regeneration of tropical forests. Additionally, the significance of arthropods in the diet of frugivourous bats during times of fruit scarcity can also be clarified using genetic analyses. A related research question is to assess differences in fruit nutritional values

between forest matrix and river bank vegetation, since light and ambient conditions vary dramatically between the habitats and their discontinuities.

Finaly, the availability of LiDAR - Light Detection and Ranging – portable instruments can help in the quantification of vegetation clutter and the potential constrains that it imposes on flying vertebrates in the complex multi-layered rain forests. This new tool can aid researchers to assess with precision the role of vegetation clutter on foraging by those vertebrates and how it works as an ecological filter. Species specific responses to this filter can be evaluated with ecomorphological analyses that include parameters of bat wing morphology and manoeuvrability published recently (Marinello and Bernard 2014, Farneda *et al*. 2015). These models, together with the structure of echolocation calls, will help us to predict responses of bat species to changes in forest structure.

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